N68-13776

"Clinical Nutritional Study of
Minimum Protein and Caloric
Requirements for Man"

ANNUAL REPORT
to
NATIONAL AERONAUTICS AND
SPACE ADMINISTRATION

NASA Grant NGR-05-003-068

for period covering

September 1965 to September 1966

submitted
by

Sheldon Margen
Professor of
Human Nutrition

Doris Howes Calloway
Professor of Nutrition

DEPARTMENT OF NUTRITIONAL SCIENCES
UNIVERSITY OF CALIFORNIA, BERKELEY
CALIFORNIA 94720

## *Preface*

Responsibility for the conduct of experiment number 6 was carried in large part by Mrs. Janet Dale Bell and her Master's thesis is incorporated, with additions, into this report. As always, the total effort involved many persons. These were:

Administration:
Amy C. Odell, Ph.D.

Penthouse Staff:
Doris Armstrong, R.N.
Evelyn Peratrovich, R.N.
Sarah Long, R.N.
Geraldine Haugen, R.N.
Marta Miller
Arthur Miller

Dietary Staff:
Janet Dale
Jean Kincaid, M.S.

Daily Exercise Program:
Doris Armstrong

Energy Expenditure Studies:
Robert Bradfield, Ph.D.
Patricia Beard

Body Measurements and Composition:
Joseph Royce, Ph.D.
Amy C. Odell
Doris Armstrong

Biochemistry:
Robert Sandman, Ph.D.
Janet Dale
Francois Costa
Melinda Buchanan
Bert Christensen
Bernadette Couly
Delroy Brown
Gwendolyn Jackson

Data Reduction and Analysis:
Ruth Friedman

Secretary:
Marjorie Teach

The administrative staff of the Department of Nutritional Sciences gave us excellent support as always.

We wish to thank all of them, as well as the enthusiastic and cooperative subjects and the local physicians who referred them to us.

Doris Howes Calloway

Sheldon Margen

## CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## INTRODUCTION

Systems proposed for regeneration of cabin atmosphere in prolonged space flight and planetary stations are most effective when they serve a dual role, as sources of both oxygen and food. Presently conceived chemical systems produce in high yield only carbohydrates or carbohydrate-like substances and require that supplies of protein or amino acids be stored or regenerated by a second, complementary system. Thus, a first limitation of these schemes would appear to be the minimum amount of protein that must be provided. (The minimum requirement for essential fatty acids is reasonably well established and can be met easily from less than one ounce of common oils.) Establishment of this minimum has formed the topic of our earlier studies under this grant.

On the other hand, in systems involving a biological agent as the regenerator, high growth rate of the agent is required and the cellular product thus includes high concentrations of protein and of genetic material, nucleic acids. These systems might require supplementation with additional non-protein energy sources if the protein yield exceeds man's tolerance for this nutrient form. If a supplementary energy source were to be carried, fat would be the more desirable form because it yields more calories per unit weight and volume than does carbohydrate. Thus, both the maximum tolerance for protein and the minimum need for carbohydrate must be accurately known.

It is not generally agreed that there is a requirement for carbohydrate, as such, in the diet, since hexose can be manufactured in the body from glycerol and from some of the amino acids. There is no question that carbohydrate is required at the tissue level. The function of the nervous system normally depends upon a supply of glucose (although recent evidence indicates that brain tissue can use an intermediate of fat metabolism (1)). Metabolically, a supply of carbohydrate is necessary to permit acetate (from fat oxidation and other precursors) to enter the citric acid cycle. In the latter case, the carbohydrate provides the needed pyruvate and oxaloacetate that are not fully regenerated in the cycle. When fat oxidation is the dominant source of energy and carbohydrate is lacking (as in fasting or uncontrolled diabetes), the rate of formation of carbohydrate intermediates from precursors may be inadequate to allow complete utilization of acetate. The acetate residues then condense to form the ketone bodies, acetoacetic acid and beta-hydroxybutyric acid. These substances are normally formed in small amounts and can be utilized to some extent by the body tissues, but when they are present in excess, they accumulate in the blood and are excreted in the urine. By decarboxylation, acetone is formed and this end-product is often detected in the breath of ketotic subjects.

Excretion of these incompletely burned metabolites in the urine does constitute some loss of potential energy (energy value is about 7 kcal/g), but, more importantly, ketone bodies are strong acids and to buffer them the alkali reserve will be depleted. Ultimately, acidosis and dehydration will result. Another undesirable feature of ketosis is that renal tubular secretion of uric acid (the end-product of metabolism of the purine base of nucleic acids) is suppressed and the uncleared uric acid accumulates in the blood(2). Acute episodes of gout, i.e., ~~ketosis~~s, elevation of serum uric acid, and deposition of uric acid crystals in the joints, have been precipitated during fasting in the obese (3).

There is also evidence that carbohydrate is necessary specifically for the retention of sodium (4). A significant fraction of the energy expenditure of the kidney is involved in sodium transport and the medullary portion of the kidney uses glucose for energy production (5). ¶ The only long-term study of men consuming a calorically adequate diet nearly devoid of carbohydrate was that of the explorers Stefansson and Andersen who lived exclusively on meat and animal tissues for one year. Their average daily intake was 2500 to 3000 kcal, of which about 500 kcal was from protein. E. Tolstoi (6) reported that blood sodium, glucose, non-protein nitrogen, and uric acid remained normal and that there was no evidence of illness even though ketonuria occurred continuously with averages of 0.4 to 7.2 g of total ketone bodies excreted daily. In a more detailed account of the ketosis in the same two subjects, W. S. McClellan and E. F. Dubois(7) state that acetone appeared in the urine on the very first day and increased until the fourth day, after which it was constant in one subject and gradually decreased in the other. Ketonuria persisted when 5% or less of the calories was from carbohydrate (approximately 35g) but promptly disappeared when 200 g carbohydrate, 30% of the daily caloric intake, was fed to the subjects. In this study, protein intake was not high (about 125 g/day), so uric acid precursors were relatively limited, as compared with a diet based on a large amount of lean animal tissue or bacterial cells.

It is well known that carbohydrate has a protein-sparing effect. J. L. Gamble reported that young men who fasted for 6 days lost 400 g of body protein (8). When they ingested 50 g glucose a day, they lost 325 g of body protein in 6 days and when the carbohydrate was increased to 100 g glucose, a maximum sparing effect resulted, with a loss of only 240 g body protein in 6 days. In other words, ingestion of 100 g of glucose a day resulted in the reduction of body protein loss to 40 g/day from 67 g/day protein loss in fasting. This amount of carbohydrate also prevented ketosis and sodium loss.

The addition of fat to the diet during inadequate protein intake does not have the same protein-sparing effect as carbohydrate. It was noted by E. P. Cathcart (9) that when carbohydrate was wholly replaced by fat in a diet containing 3 g nitrogen per day, the urinary nitrogen output rose decidedly. This accelerated loss of body protein does not appear to happen on protein-free diets, provided a certain minimum of carbohydrate is still ingested. Munro (10) says that the mode of action of this protein-sparing is not clear but it appears to be the result of two mechanisms, one related to the level of energy intake and one specifically connected with carbohydrate intake.

Significant areas of question thus seem to concern ability to metabolize without hazard various amounts of nucleic acid-containing protein in diets providing fat as the dominant energy source and with little or no preformed carbohydrate. Planning for long-term flight feeding thus requires sequential determination of the minimum need for carbohydrate; the maximum tolerance for nucleic acid-free protein with and without carbohydrate and fat; and, finally, the tolerance for nucleic acid in diets of varying composition. As a forerunner of systematic studies of normal men, we conducted a study of obese subjects given very low calorie diets composed exclusively of single energy sources--egg albumin, corn oil and sucrose--or combinations of these with adequate vitamins and minerals. Under these conditions, the dominant energy source at the tissue level may be presumed to be fat and, to varying degrees, lean tissue of the body. Our purpose was to discover whether exogenous protein in amounts normally required could serve as a sufficient precursor of carbohydrate, sparing tissue protein and preventing ketosis, i.e., whether a specific need for carbohydrate exists when the endogenous energy reserve is abundant, or whether protein will suffice. The incorporation of an all-fat diet allowed an estimate of whether or not exogenous calories were beneficial when the organism has already available a large amount of the same substrate, fat. The data on protein feeding were compared with results obtained in other subjects whose body weights were in the upper range of the population normal average for their heights and ages (i.e., 5 to 10 kg above ideal body weight).

## SUMMARY AND CONCLUSIONS

As an initial step in determining if there is a requirement for carbohydrate, as such, in the diet, a formal Penthouse experiment was carried out (Penthouse Study #6) and a brief informal test was made of one point in a few unconfined normal subjects. In the major study, five obese women were fed for six sequential 5-day periods diets providing 400 kcal from egg albumin, corn oil or sucrose and 800 kcal from 50:50 mixtures of egg albumin and corn oil or sucrose. One intended dietary treatment involving 400 kcal as the glucogenic amino acid, glycine, could not be completed because it induced nausea and vomiting in most of the subjects. Two of the subjects were unable to tolerate a second administration of the fat formula in conjunction with the egg albumin mixture so the replications were not complete for this 800 kcal treatment. These women were given 400 kcal as a 50:50 mixture of egg albumin and sucrose instead, to judge whether 50 grams of carbohydrate would be as effective as 100 grams proved to be.

Mean weight loss for the 30 days of the experimental dietary treatments was 10.54 kg. In four of the subjects, loss was greatest when fat was included in the diet, with or without protein, and least in the presence of carbohydrate. These differences in weight were associated with mineral excretion patterns suggestive of retention of extracellular fluid when carbohydrate was included in the diet and loss or shifts among compartments when fat was present. The greatest losses of weight were also associated with periods in which ketosis was most severe and were accompanied by loss of lean tissue from the body, based on observed nitrogen balances. Ketosis was most severe with the all-fat diet. Urinary excretion of uric acid fell and serum level rose in conjunction with fat and protein treatments devoid of carbohydrate. Fasting blood glucose levels were higher when the diet included carbohydrate than when fat was given. In most respects, protein was intermediate between carbohydrate and fat in its effects on weight loss and metabolic patterns. Addition of 400 kcal exogenous fat to a diet providing 400 kcal from egg albumin did not significantly alter the response obtained when the albumin was given as the sole energy-yielding substance.

Provision of only 400 kcal as egg albumin (80+ grams of protein and carbohydrate residues from muco-proteins) did little to prevent the loss of lean body tissue, in comparison with 400 kcal from sucrose, but it was an improvement over 400 kcal of exogenous fat. (One obese subject did achieve nitrogen balance at the low level of protein intake, but this occurred only after two preceding irregular periods: one of semi-starvation due to an attempt to consume the glycine formula, followed by a period in which protein was consumed without the prescribed mineral supplement.) When the diet included 400 kcal from carbohydrate in addition to the 400 kcal from egg albumin, two subjects were in positive nitrogen balance and balance was less negative in two others. Addition of fat was less advantageous than provision of protein alone.

Additional data on this point were gathered in short-term tests of the response of normal but somewhat overweight subjects to consumption for 5 to 12 days of a pure protein, casein, as the energy-yielding component of diets providing nearly 400 kcal daily. One other subject took 400 kcal as egg white, crab or turkey for 5 days and 400 kcal as carbohydrate in a second 5-day period. The response of these subjects differed from the obese only in that urinary nitrogen output was higher and body protein loss greater than in obese women. In the one subject for whom comparative data were available, there was no evidence that dietary protein protected against loss of tissue protein. In the normal subjects, as in the obese, serum uric acid levels were elevated with protein feeding and blood urea nitrogen content was disproportionately high, relative to nitrogen intake.

These findings support, in general, conclusions reached earlier with respect to the response of nonobese young men given low-calorie diets containing variable amounts of protein (11) that, at very low levels of caloric intake, exogenous protein is burned chiefly as an energy (or carbohydrate) source. The findings with the 800 kcal protein plus carbohydrate diet are consistent with the earlier conclusion that about 900 kcal is the lowest level at which dietary protein reliably and effectively spares tissue protein, beyond provision of the same amount of nonprotein energy derived from mixtures containing some carbohydrate.

Presumably, the glucogenic function of protein would vary with its proportional content of glucogenic and ketogenic amino acids. In the one subject who completed the glycine treatment, ketosis and mineral excretion patterns were essentially the same as with carbohydrate, but urinary nitrogen output was high and tissue protein was not spared. There is some indication, from only two subjects, that 50 grams of sucrose (200 kcal) with 200 kcal from egg albumin is as effective as 100 grams of sucrose alone (400 kcal) in preventing most of the sequellae of carbohydrate deprivation at low calorie levels.

The Penthouse study also affords some evidence of systematic adaptation to continued subsistence on calorically inadequate diets. These were: decreased rate of weight loss and, perhaps secondarily to loss of body mass and lean tissue, decreased creatinine excretion, decreased catabolism of lean tissue and reduced energy cost of active work. Serum total cholesterol fell sharply at the beginning of the test, remained stable for some time, and showed evidence of an upswing at the end.

An interesting sidelight of the study was the observation that adipose tissue did not behave fully as an organ, because proportionately more tissue was removed from some body sites than others (most from the upper arm, thigh and neck and least from the chest, waist and hips), as measured by change in body dimension.

We conclude that carbohydrate is necessary in the diet to prevent ketosis and loss of cations, to spare tissue protein and to promote adequate clearance of uric acid, when endogenous fat is the chief source of energy. Exogenous protein can fulfill part of this function, but it is less effective than carbohydrate at equivalently low caloric intakes.

## PROCEDURE

### A. PENTHOUSE EXPERIMENT NO. 6

The 5½-week experiment involved study of 6 obese women residing in the Human Research Laboratory of the Department of Nutritional Sciences, University of California, Berkeley, where they were fed single-nutrient formula diets at levels of either 400 or 800 kcal/day. A preliminary 4-day period of stabilization on a balanced formula diet providing 2000 kcal/day was followed by six 5-day metabolic periods on the single-nutrient diets and a final 4-day period on a balanced diet providing 900 kcal/day, given on the first 2 days as "Metrecal"* and on the last 2 days as ordinary food. Excreta were collected for determination of external nitrogen balance. Ketone bodies were measured quantitatively in urine and blood, as was uric acid and other relevant constituents.

#### *Selection of Subjects*

Six subjects were chosen, after medical examination, from obese volunteers referred for study by local physicians. Female subjects were selected because there were too few male volunteers to form an experimental group. After the first week of the project one subject left of her own volition. Results are therefore presented for the remaining 5 subjects.

One subject was 17 years old; the others were between 37 and 55 years. Weight ranged from 116 to 141 kg (256 to 310 lbs) at the beginning of the study. One subject was mildly diabetic, adequately controlled with an oral hypoglycemic agent, without insulin. The others were free of known systemic disease and all had normal blood chemistry and cellular characteristics. Case histories of these 5 subjects are given in Appendix A.

#### *Diets*

We planned to use 6 experimental dietary treatments. The 4 basic diets provided 400 kcal/day from a single one of the following energy sources: sucrose (C = carbohydrate); corn oil** (F = fat); egg albumin*** (P = protein);

---

*A proprietary liquid formula intended for low-calorie diets. Mead-Johnson & Co., Evansville, Ind.

**Mazola, Corn Products Company, N.Y.

***Carlson's Bakers Supplies, Oakland, Calif. At the 400-kcal level, egg albumin provided 80 g of protein.

or glycine* (G). The remaining 2 diets provided 800 kcal/day of which 400 kcal was derived from the basic egg albumin diet in both cases, and the other 400 kcal came from the basic carbohydrate diet in one case and from the basic fat diet in the other, administered in conjunction with the protein.

The original test design provided that every subject was to receive the diets in a different sequence, in order to nullify the effects of the preceding diet on the overall results for any one test diet. No two subjects were to be on the same diet during any one period. However, changes had to be made due to the difficulties some subjects had in tolerating certain diets. Only one subject (0601) completed all 6 dietary periods as originally planned. It was discovered at the beginning of each metabolic period that the glycine diet caused vomiting and discomfort in each subject in turn, although Subject 0601 managed to retain all her meals throughout that 5-day period. After the fourth period, attempts to administer the glycine diet were abandoned and a 50:50 diet of protein and carbohydrate at 400 kcal/day was substituted in its place. During the final metabolic period Subject 0602 was no longer able to tolerate the fat formula, and a second 400 kcal/day of protein was substituted in place of the 400 kcal of fat in the 800-kcal/day fat + protein diet. In the fifth period, 400 kcal/day of fat was omitted from the diet of Subject 0605 for the same reason. Table 1 shows the order in which each subject was actually fed the test diets.

Tables 2, 3, 4, and 5 show the constituents of the 4 basic test diets and the manner in which they were prepared. Five g of agar** per 400 kcal was used in all the diets to provide a semi-fluid consistency and to prevent separation of the mixture on storage. The carbohydrate diet included a small amount of citric acid to decrease the sweet taste. Calcium cyclamate*** was used to sweeten the other diets. Since raw egg white contains avidin, a protein that binds biotin, 200 micrograms of biotin per 100 g of egg albumin was added in the diet preparation to counteract the avidin present.

Salts were added to provide approximately the following amounts of minerals during all of the 6 metabolic periods, in g/subject/day: sodium, 3.0; potassium, 3.0; calcium, 0.8; phosphorus, 1.2; magnesium, 0.5.

---

*Monoaminoacetic acid, Nutritional Biochemicals Corp., Cleveland, Ohio.
**"Bacto-agar," Difco Labs, Detroit, Mich.
***Sucaryl, Abbot Laboratories, North Chicago, Ill.

Table 1

DIETARY PLAN

Experiment No. 6

| Metabolic Period → | Pre | I | II | III | IV | V | VI | Post | |
|---|---|---|---|---|---|---|---|---|---|
| Number of Days → | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 2 | 2 |
| | | | | | Diet | | | | |
| **Subject** | | | | | | | | | |
| 0601 | X | F+P | G | P | C | F | C+P | Y | Z |
| 0602 | X | C | F | G(1) | P | C+P | 2P | Y | Z |
| 0603 | X | P | C+P | F+P | F | ½(C+P) | C | Y | Z |
| 0604 | X | F | P | C+P | F+P | C | ½(C+P) | Y | Z |
| 0605 | X | C+P | C | F | G(2) | P | P | Y | Z |

| Diet | Content | Total kcal/day |
|---|---|---|
| X | NASA 5112 - Balanced Formula* | 2000 |
| G | Glycine | 400 |
| F+P | Corn oil (400 kcal) + egg albumin (400 kcal) | 800 |
| C | Sucrose | 400 |
| C+P | Sucrose (400 kcal) + egg albumin (400 kcal) | 800 |
| P | Egg albumin | 400 |
| F | Corn oil | 400 |
| ½(C+P) | Sucrose (200 kcal) + egg albumin (200 kcal) | 400 |
| 2P | Egg albumin | 800 |
| Y | "Metrecal" | 900 |
| Z | Normal low-calorie diet | 900 |

(1) First 2 days essentially fasting + 3 days of 400 kcal carbohydrate/day.

(2) First 2 days essentially fasting + 3 days 400 kcal carbohydrate/day; no minerals throughout period.

* Formula will be found in annual report dated September 1966.

Table 2

COMPOSITION AND PREPARATION OF CARBOHYDRATE DIET

| | g/day | |
|---|---|---|
| Sucrose | 102.72 | (397.53 kcal) |
| Citric acid | 1.0 | (2.47 kcal) |
| MgO* | .759 | |
| $CaHPO_4\ 2H_2O$* | 3.35 | |
| KI* | .002 | |
| Agar | 5. | |
| $H_2O$ (1) | 250. | |
| $H_2O$ (II) | 300. | |
| Flavoring, artificial | .02 | |
| Coloring, artificial | .02 | |

*Omitted from carbohydrate diets prepared for administration with protein diets.

Prepared by mixing dry ingredients together with some $H_2O$ (II).
The agar was dissolved by bringing to boil in $H_2O$ (I) with constant stirring, allowing it to cool, and making up to the original weight with water before mixing all ingredients together.
Weighed into separate meal containers (164.7 g/100 kcal meal) and frozen.
Sufficient diet for the complete project was made at one time.

Table 3

COMPOSITION AND PREPARATION OF FAT DIET

| | g/day | |
|---|---|---|
| Corn oil | 45.25 | (400 kcal) |
| "Sucaryl" | 1.0 | |
| MgO* | .759 | |
| $CaHPO_4\ 2H_2O$* | 3.35 | |
| KI* | .002 | |
| Agar | 5. | |
| $H_2O$ (I) | 250. | |
| $H_2O$ (II) | 250. | |
| Flavoring, artificial | .04 | |
| Coloring, artificial | .03 | |

*Omitted from fat diets prepared for administration with protein diets.

The agar was dissolved by bringing to boil in $H_2O$ (I) with constant stirring, allowing it to cool, and making up to original weight with water.
The dry ingredients were suspended in a little $H_2O$ (II) and all ingredients were blended in a Waring Blender, chilled in the freezer for 20 minutes, briefly reblended, weighed into separate meal containers (137.8 g/100 kcal meal) and frozen.
Sufficient diet for 6 subject-days was made at one time.

Table 4

COMPOSITION AND PREPARATION OF PROTEIN DIET

| | g/day | |
|---|---|---|
| Egg albumin | 107.4 | (400 kcal) |
| "Sucaryl" | 1.25 | |
| Biotin | .0002 | |
| MgO | .618 | |
| $CaHPO_4\ 2H_2O$ | 3.19 | |
| KI | .002 | |
| Agar | 5. | |
| $H_2O$ (I) | 250. | |
| $H_2O$ (II) | 750. | |
| Flavoring, artificial | .05 | |
| Coloring, artificial | .03 | |

The dry ingredients were mixed with a little $H_2O$ (II) into a thick paste and gradually thinned with $H_2O$ (II), avoiding any beating motion (which would have caused persistent foaming).
The agar was dissolved by bringing to boil in $H_2O$ (I) with constant stirring, allowing it to cool, and making up to original weight with water before gently mixing all the ingredients together.
Weighed into separate meal containers (279.4 g/100 kcal meal).
Sufficient diet for one-quarter the complete project was made at one time.

Table 5

COMPOSITION AND PREPARATION OF GLYCINE DIET

| | g/day | |
|---|---|---|
| Glycine | 107.5 | (400 kcal) |
| "Sucaryl" | .25 | |
| MgO | .759 | |
| $CaHPO_4\ 2H_2O$ | 3.35 | |
| KI | .002 | |
| Agar | 5. | |
| $H_2O$ (I) | 250. | |
| $H_2O$ (II) | 1000. | |
| 12N HCl | .320 | |
| Flavoring, artificial | .120 | |
| Coloring, artificial | .040 | |

The agar was dissolved by bringing to boil in $H_2O$ (I) with constant stirring, allowing it to cool, and making up to the original weight with water.
The dry ingredients were mixed together in a little of $H_2O$ (II) and all the ingredients were well mixed before weighing into separate meal containers (341.8 g/100 kcal meal) and freezing.
Sufficient diet for the complete project was made at one time.

Sodium was given in the form of sodium chloride tablets and potassium as a separate liquid supplement, since at the desired levels they would have made the diets completely unpalatable. The other salts, which are tasteless, were mixed into the diets. Because egg albumin already contains certain amounts of minerals, two different levels of sodium chloride (4.5 g/day with the protein diet; 7.5 g/day without the protein diet) and of potassium supplement (see Table 6) had to be administered and the carbohydrate and fat diets had to be prepared in 2 forms, one with minerals added for use alone and one without minerals for use at the 800 kcal/day level, in conjunction with the protein diet which already contained the daily supply of minerals.

Trace minerals and vitamins were administered daily as listed in Tables 7 and 8.

Each subject received daily 4 cups of decaffeinated coffee* (9.0 g), 1 cup of tea** (1.0 g), and one bottle of artificially sweetened carbonated beverage*** (12 oz., approximately 290 g). Subject 0602 received 4.0 g tea daily in place of coffee throughout the project and was given an extra daily fluid mineral supplement to replace the minerals thus omitted.

In addition to the diets already described, the subjects had free access to deionized water from a cooler at all times. Each subject recorded all volumes (in ml) of self-administered water on her individual chart. No other materials were permitted by mouth, including toothpaste, mouthwash, or chewing gum, unless administered by a staff member.

The basic diets were prepared in bulk in advance of the project, weighed into individual meal containers, each of which held a 100-kcal portion, and frozen until needed. Shortly before administration the containers were defrosted and one or two 100-kcal portions were given at 9:00 a.m., 12:30 p.m., 5:30 p.m., and 9:30 p.m. each day. The subjects drank the semi-fluid formulas straight from the containers and were instructed to clean the containers thoroughly with spatulas provided. One of the investigators checked each carton for complete consumption.

---

*Sanka, General Foods, Institutional Food Ser. Div., White Plains, N.Y.
**Nestea, The Nestle Co. Inc., White Plains, N.Y.
***Tab, Coca Cola Company.

Table 6

LIQUID POTASSIUM SUPPLEMENTS*

1. For use with protein diet: 60 ml provides 1450 mg K

| | per 6 liters |
|---|---|
| $K_2HPO_4 \cdot 3H_2O$ | 368.2 g |
| $K_2SO_4$ | 41.9 g |
| $H_2SO_4$ (conc.) | 36.0 ml |
| Distilled water | q.s. |

2. For use without protein diet: 60 ml provides 2620 mg K

| | per 6 liters |
|---|---|
| $K_2HPO_4 \cdot 3H_2O$ | 412.4 g |
| $K_2SO_4$ | 269.2 g |
| $H_2SO_4$ (conc.) | 42.0 ml |
| Distilled water | q.s. |

*15 ml administered 4 times daily with meals.

Table 7

TRACE MINERAL SUPPLEMENT*

| Formula | | Yield | |
|---|---|---|---|
| $FeSO_4 \cdot 7H_2O$ | 50 mg | $Fe^{++}$ | 10 mg |
| $CuCl_2 \cdot 2H_2O$ | 5.37 mg | $Cu^{++}$ | 2 mg |
| $ZnSO_4 \cdot 7H_2O$ | 43.9 mg | $Zn^{++}$ | 10 mg |
| $MnSO_4 \cdot H_2O$ | 15.36 mg | $Mn^{++}$ | 4.9 mg |
| $Na_2MoO_4 \cdot 2H_2O$ | 0.63 mg | $Mo^{+++}$ | 0.24 mg |
| $Cr_2(SO_4)_3 \cdot 15H_2O$ | 3.2 mg | $Cr^{+++}$ | 0.5 mg |
| $Na_2SeO_3 \cdot$ | 25 mcg | $Se^{++++}$ | 11.0 mcg |
| $AlK(SO_4)_2 \cdot 12H_2O$ | 85.0 mg | $Al^{+++}$ | 4.8 mg |
| Choline dihydrogen citrate | 2.4g | Choline | 1 g |

*Divided into 3 capsules and given with breakfast, dinner and supper.

Table 8

VITAMIN SUPPLEMENT*, **

| | |
|---|---|
| Thiamine mononitrate | 2 mg |
| Riboflavin | 3 mg |
| Niacinamide | 20 mg |
| Pyridoxine hydrochloride | 5 mg |
| Calcium pantothenate | 10 mg |
| Folic acid | 0.5 mg |
| Vitamin $B_{12}$ | 2 mcg |
| d-biotin | 0.05 mg |
| Vitamin A acetate or palmitate | 4000 U.S.P. units |
| Vitamin $D_2$ | 400 U.S.P. units |
| dl-alpha tocopheryl acetate | 35 mg |
| Vitamin $K_1$ | 1 mg |
| Ascorbic acid | 50 mg |

*Given daily with breakfast.

**Courteously supplied by the Hoffman-LaRoche Company.

*Penthouse Procedure*

The subjects were admitted to the Penthouse Research Unit in the afternoon of Monday, May 23, 1966 (project day 1), and after introduction to test procedures were given a normal dinner. Formula diets were started on project day 2.

Urine voided at 8 a.m. the next morning was discarded and all urine was saved throughout the 38 days of the project, each 24-hour collection ending at 8 a.m. Volume, specific gravity, osmolality, and pH were measured daily and qualitative tests for sugar, protein, and acetone were made. Daily collections were diluted to convenient volume with distilled water and aliquots were analyzed for creatinine, nitrogen, and total ketone body contents. Pooled collections from each 5-day metabolic period were analyzed for content of uric acid, sodium, and potassium. Methods used for these and other chemical procedures are listed in Appendix B.

Fecal collections were made for each 5-day metabolic period, beginning on project day 5. The weight of stools was recorded at each defecation. The 5-day collection was diluted to uniform volume with distilled water, blended thoroughly in a colloid mill, and aliquots were analyzed for nitrogen content.

A fasting blood sample was taken on the first day of each metabolic period, and the day after period VI ended. These were analyzed for total ketone bodies, total nitrogen, urea nitrogen, uric acid, glucose and cholesterol.

The subjects were weighed to the nearest 10 g each morning on rising and immediately after voiding, clad only in underwear. Body composition was estimated for 2 subjects from specific gravity (underwater weighing), before the first metabolic period and during the sixth period. Body composition was estimated for all subjects from total body water ($T_2O$) during the first metabolic period and at the end of the sixth. Body circumference measurements were made on the first day of each metabolic period, primarily for the personal satisfaction and encouragement of the volunteer subjects.

Basal metabolic rate was measured 3 times during the experiment; before the first metabolic test period, during the third, and after the sixth. Circulating blood volume was measured twice, at the beginning and at the end of the project.

Oral temperature, pulse and respiration rates, and blood pressure were recorded twice daily, on rising at 7:30 a.m. and at 9:00 p.m. before the evening meal. A breath sample was taken from each subject every morning immediately after rising, for a separate study.

Since the subjects were confined to a relatively small area and their normal exercise was restricted, a daily activity program was planned to encourage the women to use exercise to complement the anticipated weight loss from their restricted caloric intakes. As the subjects were all considerably overweight and unaccustomed to an active life, this program could not be very strenuous. Led by the nurse, the group participated in 15 minutes of simple "spot-reducing" exercises each morning before breakfast. (See Appendix C.) Every afternoon each subject walked on a treadmill, initially set level and at 2 mph. The speed and duration of walking were regulated throughout the project according to individual capabilities. It was hoped to keep activity as uniform as possible, and all subjects cooperated well except 0602, who omitted or did not complete her treadmill exercise on five occasions.

During periods V and VI both a Max Planck respirometer and conventional Douglas bag technique were used to measure energy expenditure of 4 of the 5 subjects during various daily activities. (One subject was unable to tolerate a face mask.) During this time, the subjects also kept daily records of all activity from which estimates of total daily energy expenditure were computed.

The subjects were expected to make their own beds and clean their rooms. The rest of their time was spent resting and in quiet occupations such as reading, sewing, watching television, and in conversation. There were no rules covering bedtime or sleeping but the subjects were expected to rise at 7:30 a.m. and to cooperate in all the daily procedures.

A staff member was always on duty, and a physician was available at all times. Visitors were permitted on two evenings and one afternoon each week and telephone communications and mail were allowed. The subjects left the penthouse on only two occasions during the entire project, when they were taken to be weighed underwater.

Dietary instruction was given to the subjects during the last week of the project, and on the last 2 complete project days, 37 and 38 (June 28 and 29, 1966) they were given 900 kcals per day of ordinary food to help them understand what types and amounts of food they should eat if they were to continue to lose weight when the project finished. It was intended that the subjects should return at regular intervals after the project for weight checks and encouragement.

## B. BRIEF STUDY OF NORMAL SUBJECTS

Five members of the University community, one man and four women, voluntarily consumed each day for at least 5 days 95 grams of the protein casein,* as essentially

*Casec, supplied through the courtesy of the Mead-Johnson Co., Evansville, Indiana.

the only source of dietary energy. One of the women followed the protein diet for 7 days and another for 12 days, only 9 of which were useful as she began to add some carbohydrate-containing foods on the tenth. An additional male subject took about the same amount of nitrogen, 13.1 grams per day, in the form of egg white, turkey white meat and/or crab for 5 days, and ate only dried fruits and jelly candy during a second 5-day period of 400 kcal total intake, nearly all as carbohydrate. All subjects were instructed to take two bouillon cubes daily as a source of salt, and were permitted to take *ad libitum* artificially sweetened soft drinks, coffee and tea.

These subjects ranged in age from 25 to 45 years and were about 5 to 10 kg above their desired weights for height. All were judged healthy on the basis of medical history, physical examination and normal blood values. During the test they continued their regular occupations and lived at home.

Subjects collected urine quantitatively for subsequent nitrogen determination. Blood samples drawn initially and at the end of the test diet periods were analyzed for content of uric acid, urea nitrogen, cholesterol, and triglyceride (neutral fat). Subjects weighed nude after voiding each morning, using ordinary bathroom scales.

## RESULTS

### A. PENTHOUSE EXPERIMENT NO. 6

#### *Energy Balance and Weight Change*

Daily body weights for each subject throughout experiment no. 6 are shown in Figures 1 through 5. The average weight loss for the 39 days of the whole study was 13.6 kg, the greatest loss being 15.05 kg (0604) and the smallest 12.05 kg (0605). For the 30 days of the six experimental periods the mean weight loss was 10.54 kg, with a range from 11.96 kg (0602) to 9.34 kg (0601).

Table 9a lists the weight change in grams per day by period of study. In Table 9b the same data are collated for each subject on each dietary regimen and the mean weight change for each regimen is given.

All five subjects lost weight steadily during the 4-day preliminary period of 2000 kcals mixed formula diet and stabilization had not occurred by the start of the first test period. Since this 2000-kcal diet was not appreciably lower in calories than the accepted requirements for the age and sex of the subjects, the weight loss probably represented water balance adjustment. However, weight loss rate was also greater during the first 5-day experimental period than it was thereafter, on the average. By the end of the study, subjects lost little weight even at 900 kcal/day intake, the group average being only 10% of that observed during the initial period of 2000 kcal diet.

This diminished rate of loss suggests adaptation to low caloric intakes, but there was little evidence of this in the measured basal metabolic expenditures (BME) of the subjects (Table 10). The rate of energy utilization was essentially the same in the terminal period as it was initially (within the accuracy of the method), when corrected to body mass at the date of measurement. The alteration in body weight could account for an effective difference of only about 235 kcal/day (14 kg loss x 0.7 kcal/kg/hr BME x 24 hrs). The reason for this minor change in BME may be that the dominant contributors to basal energy need--internal organs and nervous system--are little affected by short-term caloric deprivation.

It is reasonable to assume that energy cost of active tasks would be diminished as the study progressed, because the body mass to be moved was less. Very limited data obtained from four of the subjects do suggest such a systematic trend (Table 11). Early in the study, most of the subjects were unable to complete more than a few minutes of level walking on a slowly moving treadmill (1-1.5 mph), although the

Figure 1. Daily Body Weight of Subject 0601

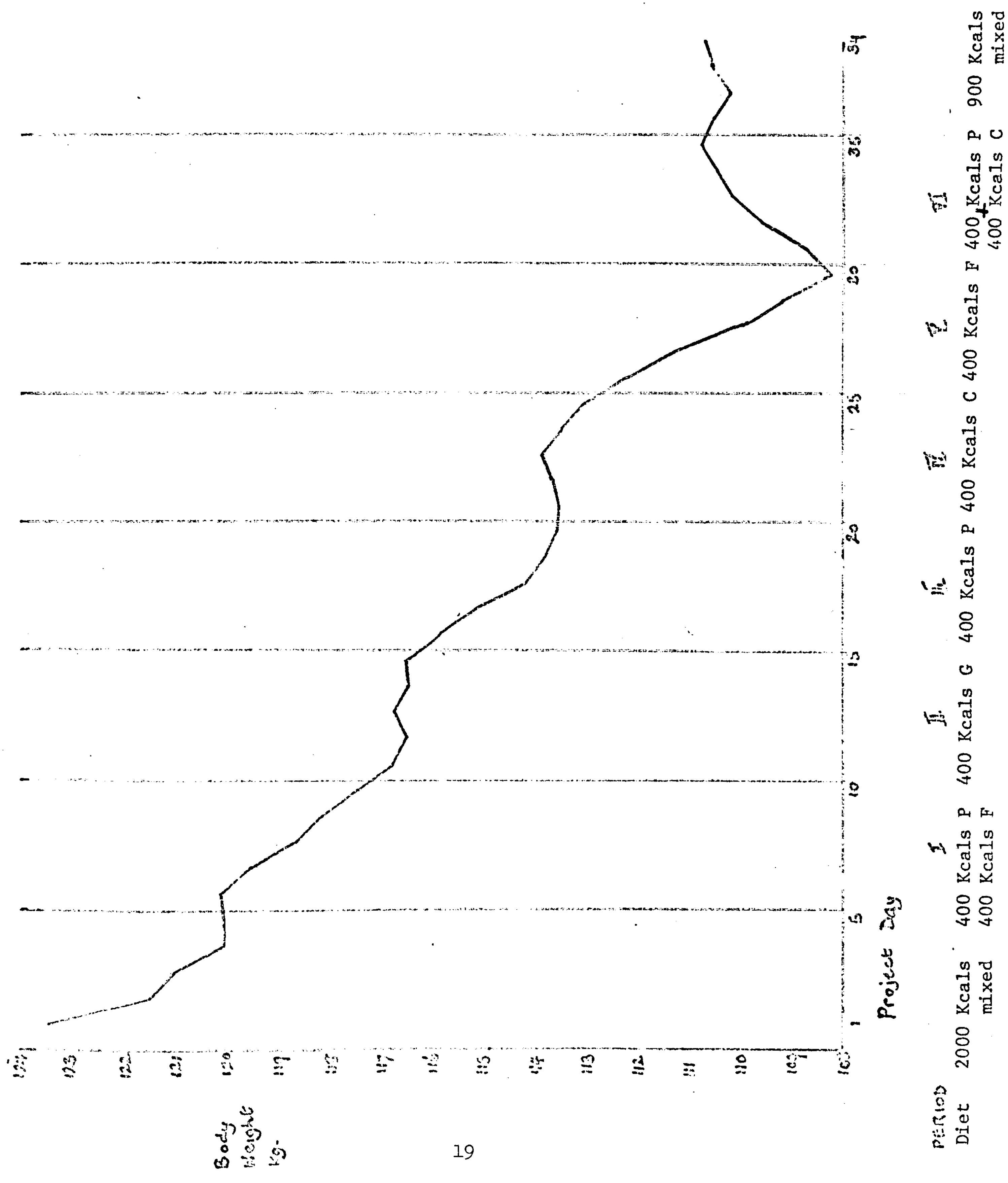


Figure 2. Daily Body Weight of Subject 0602

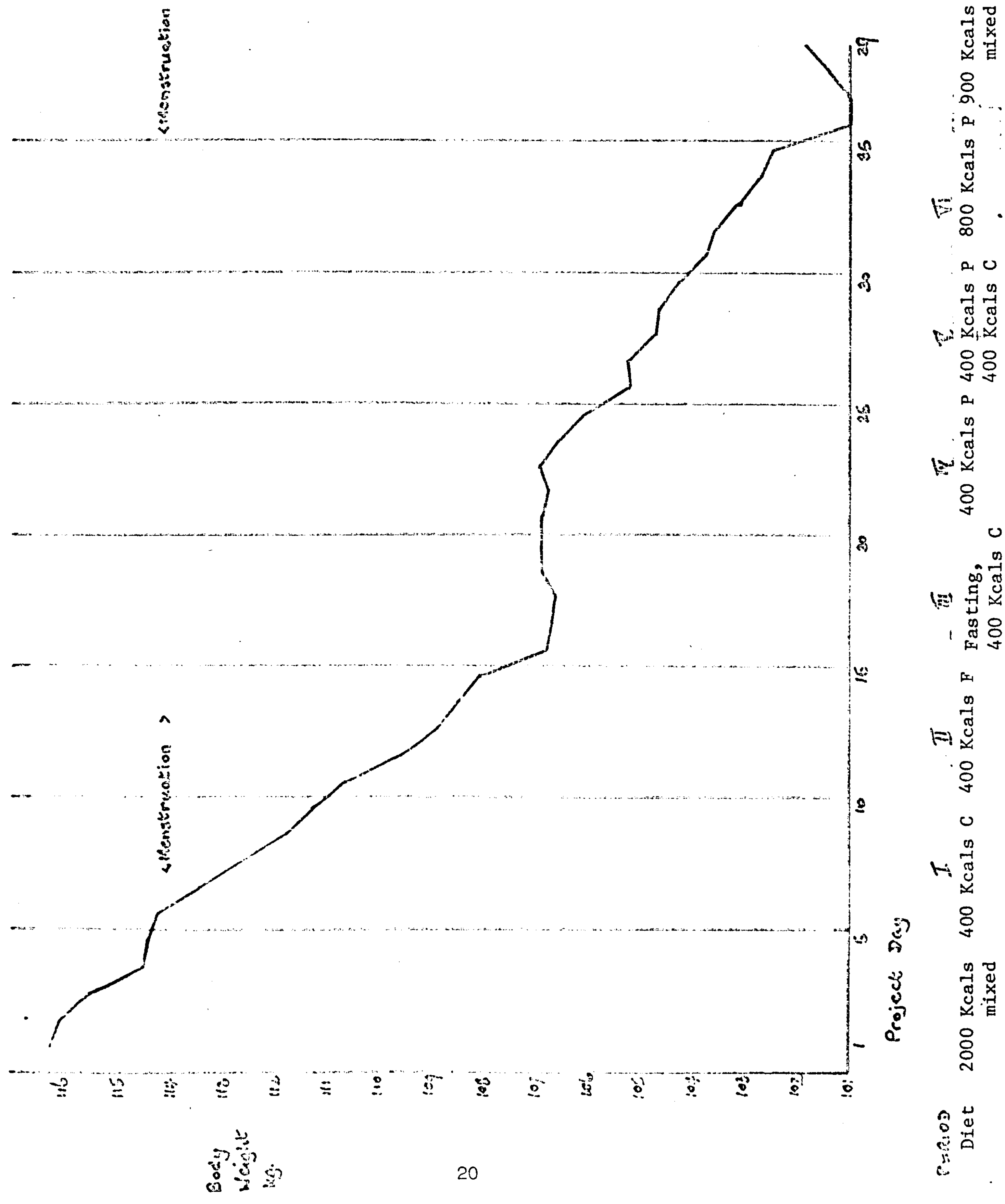


Figure 3. Daily Body Weight of Subject 0603

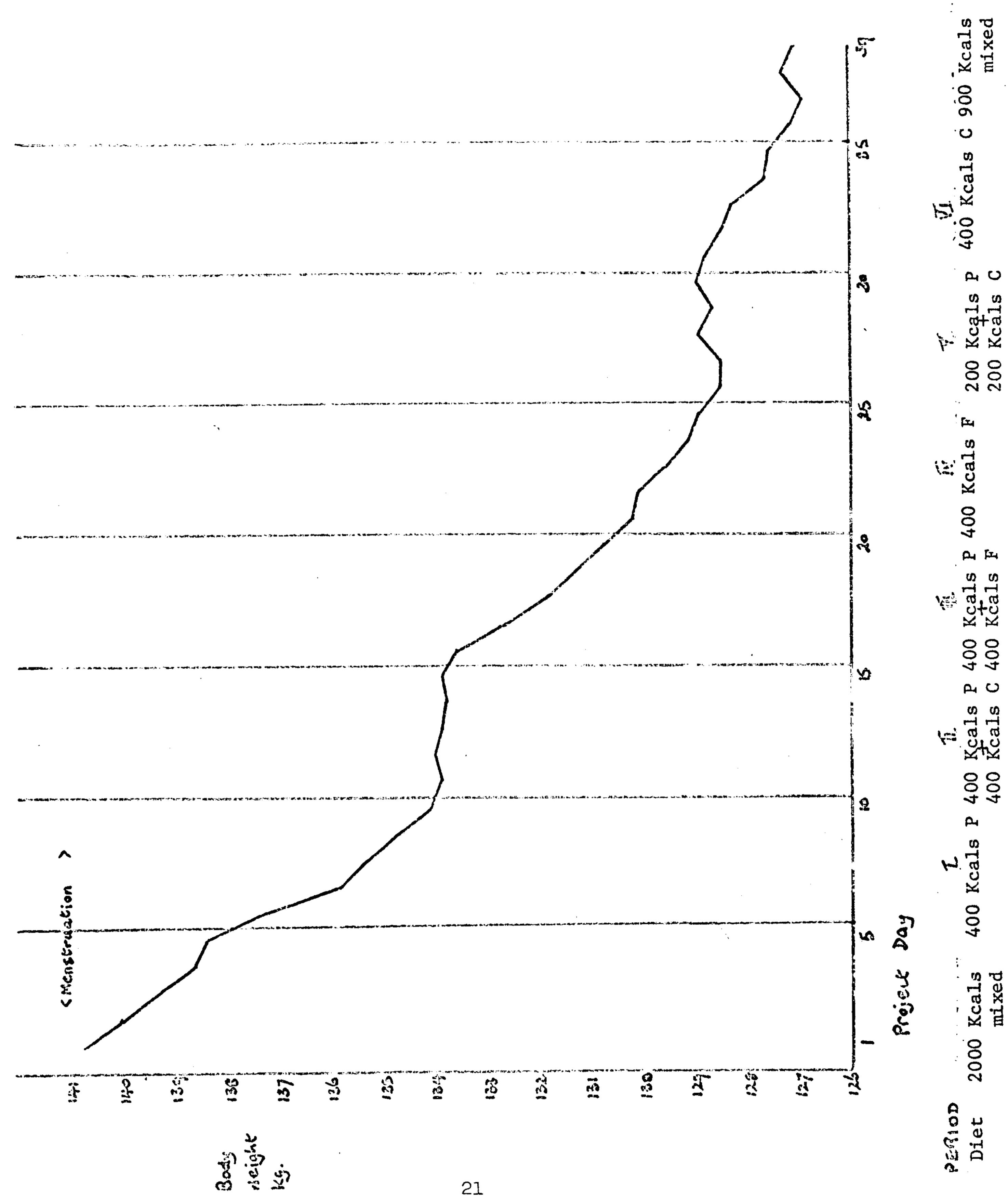


Figure 4. Daily Body Weight of Subject 0604

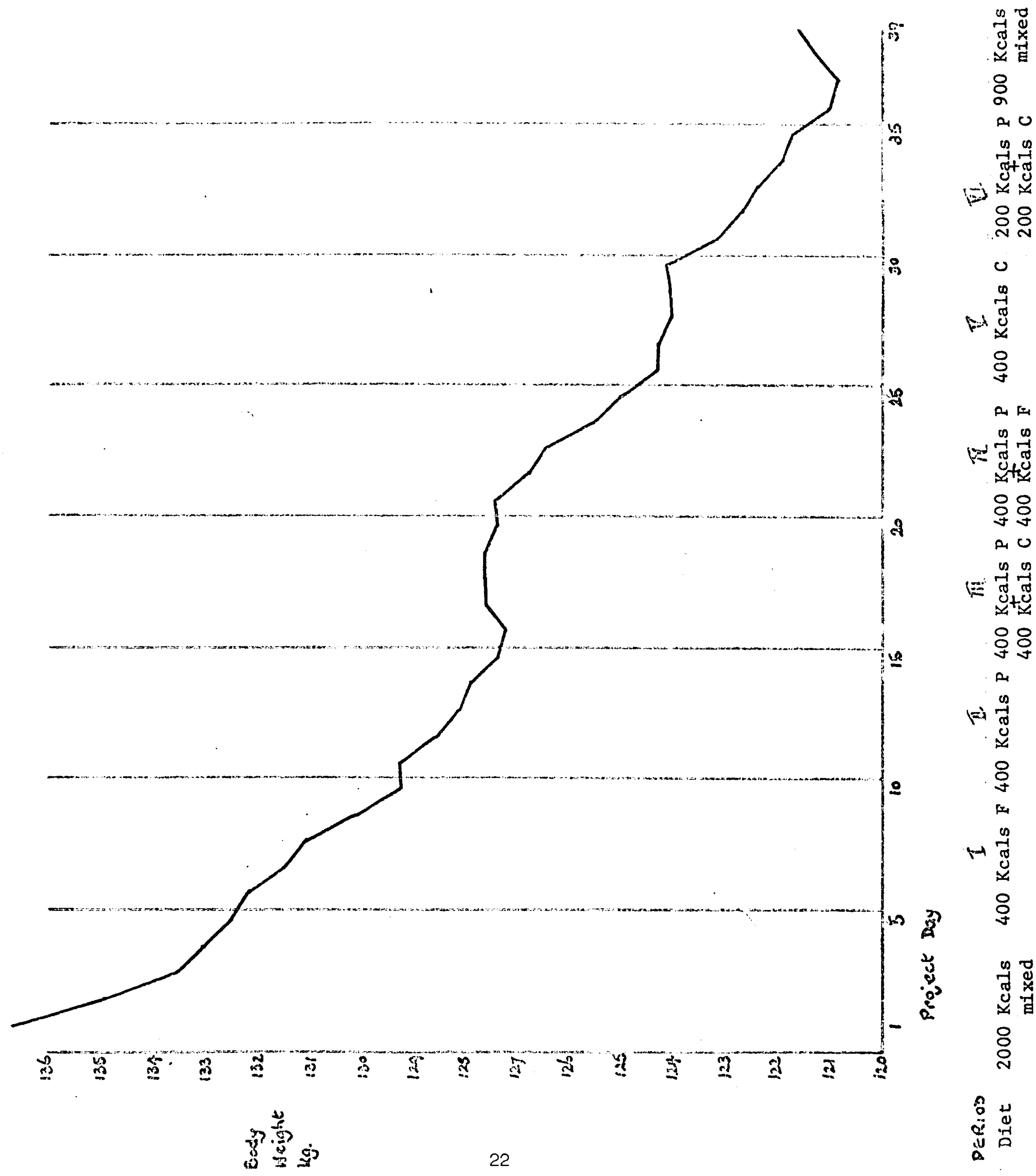


Figure 5. Daily Body Weight of Subject 0605

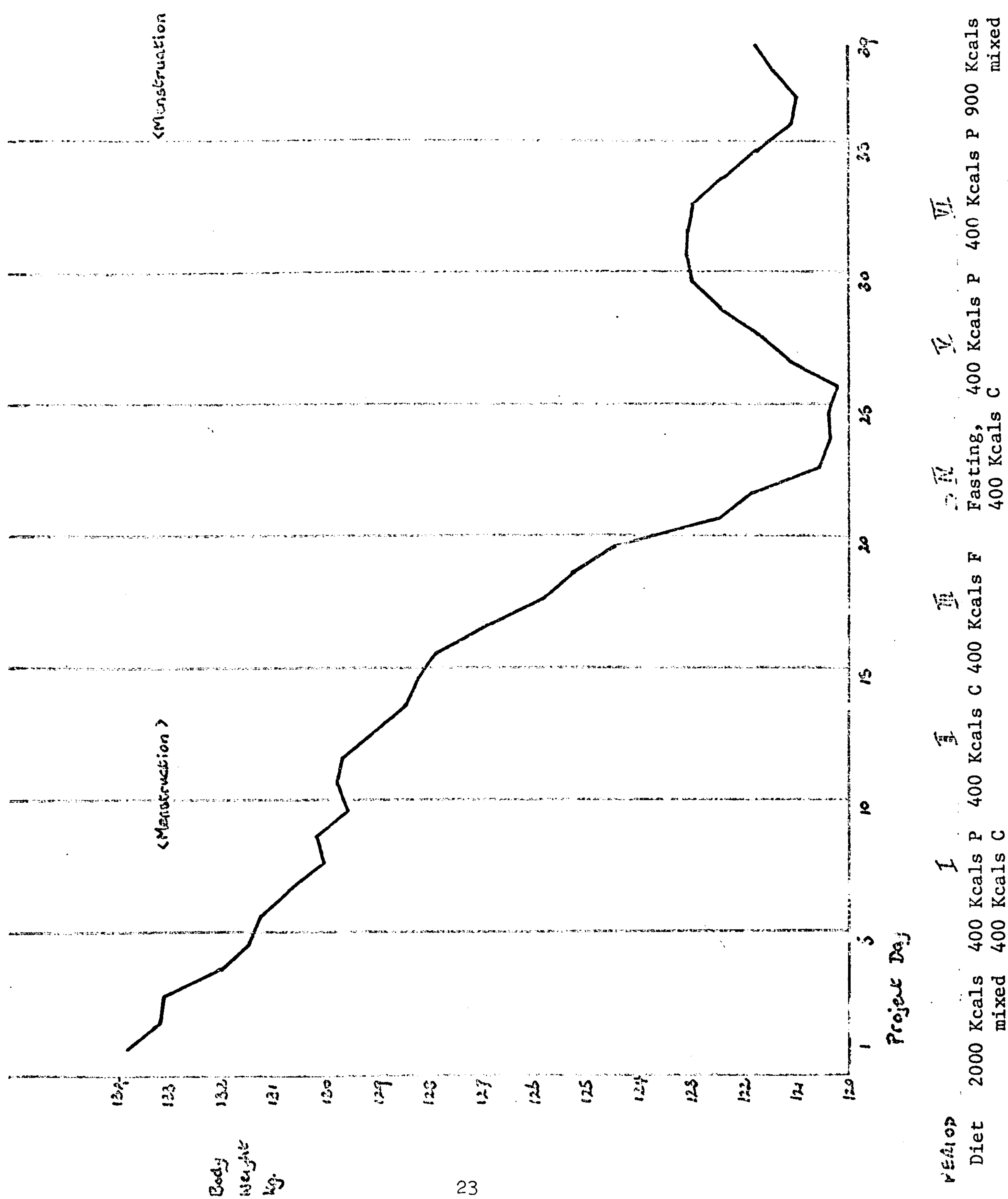


Table 9

## WEIGHT CHANGE

g/day

### A. By period of study

| Subject | (2000 kcal) Pre | I | II | III | IV | V | VI | (900 kcal) Post |
|---|---|---|---|---|---|---|---|---|
| 0601 | -835 | -524 | -190 | -592 | -112 | -956 | +506 | - 38 |
| 0602 | -458 | -626† | -648† | (1) | -174 | -352 | -358 | -162† |
| 0603 | -590† | -860† | - 52 | -628 | -358 | + 8 | -282 | -125 |
| 0604 | -1032 | -654 | -374 | + 2 | -472 | -178 | -486 | - 25 |
| 0605 | -582 | -384† | -260† | -760 | (2) | +524(3) | -244 | 0† |
| average → | -699 | -610 | -305 | -494 | -279 | -191** | -173 | - 70 |

### B. By dietary treatment

| | 400 kcal | | | | | 800 kcal | | | 2000 kcal | 900 kcal |
|---|---|---|---|---|---|---|---|---|---|---|
| | C | F | P | G | ½(C+P) | C+P | F+P | 2P | Mixed | Mixed |
| 0601 | -112 | -956 | -592 | -190 | | +506 | -524* | | -835 | - 38 |
| 0602 | -626*† | -648† | -174 | (1) | | -352 | | -358 | -458 | -162† |
| 0603 | -282 | -358 | -860*† | | + 8 | - 52 | -628 | | -590† | -125 |
| 0604 | -178 | -654* | -374 | | -486 | + 2 | -472 | | -1032 | - 25 |
| 0605 | -260† | -760 | -244(3) | (2) | | -384*† | | | -582 | 0† |
| average → | -292 | -675 | -449 | | | - 56 | -541 | | -699 | |

(1) First 2 days essentially fasting + 3 days C: -234 g
(2) First 2 days essentially fasting + 3 days C; no minerals throughout period: -820 g
(3) First 5-day period following period without minerals; weight gain presumed abnormal: +524 g
† Menses  *First period of experimental dietary treatment  **Without 0605: -369 g

Table 10

BASAL METABOLIC ENERGY EXPENDITURE

| Subject | Day of Study | Diet | kcal/hr | kcal/$m^2$/hr | kcal/kg/hr |
|---|---|---|---|---|---|
| 0601 | 2 | 2000 kcal | 99.4 | ~~9~~42.1 | 0.82 |
| | 16 | P | 115.9 | 50.8 | 1.00 |
| | 36 | Metrecal | 89.2 | 39.0 | 0.81 |
| 0602 | 2 | 2000 kcal | 80.8 | 37.4 | 0.70 |
| | 37 | Metrecal | 65.2 | 32.1 | 0.64 |
| 0603 | 5 | 2000 kcal | 89.4 | 39.0 | 0.65 |
| | 16 | F + P | 74.6 | 35.0 | 0.60 |
| | 37 | Metrecal | 84.8 | 38.2 | 0.67 |
| 0604 | 5 | 2000 kcal | 91.1 | 39.4 | 0.69 |
| | 17 | C + P | 76.0 | 33.6 | 0.60 |
| | 37 | Metrecal | 74.2 | 33.4 | 0.61 |
| 0605 | 5 | 2000 kcal | 91.1 | 40.1 | 0.69 |
| | 16 | F | 93.3 | 41.8 | 0.73 |
| | 36 | Metrecal | 80.1 | 36.5 | 0.66 |

Table 11

OXYGEN CONSUMPTION DURING VARIOUS ACTIVITIES

ml/min*

| | Period of Study | Subject 0601 | 0603 | 0604 | 0605 | Average |
|---|---|---|---|---|---|---|
| Horizontal treadmill walking | | | | | | |
| 1.6 mph | | | | | | |
| | II | | | 1060 | | |
| | IV | | 838 | | | |
| | V | 1326 | 1124 | 1324 | 1260 | 1258 |
| | VI | 928 | | 880 | 916 | 908 |
| 1.8 mph | | | | | | |
| | IV | 1456 | 1208 | 1048 | 1460 | 1293 |
| | Post | 1036 | 1024 | 994 | 964 | 1004 |
| 2.0 mph | | | | | | |
| | II | | 1620 | | | |
| | V | 1314 | 1224 | 1008 | 1460 | 1252 |
| | VI | 1076 | 1060 | | 914 | 1017 |
| 2.4 mph | | | | | | |
| | IV | 1220 | 1650 | | 1360 | 1410 |
| | V | | 1144 | 864 | 1282 | |
| | VI | 1244 | 1172 | 1132 | 1144 | 1173 |
| | Post | | | 992 | 1212 | |
| Lying quietly | | 346 | 196 | 240 | 311 | 273 |
| Sitting quietly | | 417 | 260 | 262 | 367 | 326 |
| Walking, own pace | | 737 | 485 | 817 | 890 | 732 |
| VOGUE exercises | | 787 | 562 | 537 | 550 | 609 |
| Dancing (Greek) | | | 934 | | 846 | |
| Dusting-sweeping | | | | 787 | | |

*R.Q.'s were 0.7 or less in most tests and were never above 0.8; therefore, energy value is about 4.68 kcal/liter of oxygen.

youngest, 0601, was more competent than the older women. Measured energy cost, at 2.0 mph, was about 5 to 6 kcal/min in period V (R.Q. was about 0.7 in most tests indicating dominant fat metabolism, so the energy equivalent of oxygen was about 4.68 kcal/liter), and fell to about 4.2 to 5.0 kcal/min at the end of the experiment. Young adults of normal weight (140 lbs) usually require only 2.9 kcal/min to accomplish the same work, illustrating the major energy cost of obesity, if work output is sustained.

There were remarkable differences in energy expenditure among subjects when they were allowed to work at their own pace (Table 11). The high BME of the young subject, 0601, is evident in her increased caloric expenditure during quiet sitting and lying, as compared to the older women. Expenditures during walking and exercising varied with the vigor of the subject.

Activity diaries, kept during the later periods of the experiment by some of the subjects, were used to estimate total daily energy expenditure, from the actual values of caloric expenditure for measured tasks and by interpolating for those unknown. Computed total expenditures varied widely--from averages of 1607 to 2762 kcal/day (Table 12). If these values are correct, the average daily energy deficit for the six treatment periods would be about 1650-1700 kcal/day (2188 kcal average from diaries less 520 kcal average intake from diet). Mean weight loss of the subjects in question was about 335 g/day which suggests a caloric equivalent for weight loss of about 5 kcal/g. This low value indicates that water and/or lean body mass was lost, in addition to adipose tissue (which has an energy equivalent of about 8 kcal/g based on its proximate composition).

With respect to dietary treatment, all except one subject lost most weight when given 400 kcal/day as fat; the group mean loss was 675 g/day (Table 9b). At 400 kcal, mean weight loss was least with carbohydrate (292 g). With 800 kcal from carbohydrate-plus-protein, two subjects gained weight and the mean loss was only 56 g/day. The replicates for 800 kcal from fat-plus-protein are not complete, but average loss of three subjects was 541 g, in the same range as observed at 400 kcal intakes. Thus, main dietary treatments revealed dominant effects of both carbohydrate and fat on weight loss: loss was least in the presence of carbohydrate and greatest in the presence of fat. The difference due to protein was marginal. (It is well to remember that the protein source used, egg albumen, did provide about 80 kcal from unavoidable carbohydrate present as sugar-containing protein.)

Table 12

ENERGY EXPENDITURE COMPUTED FROM DIARY RECORDS

kcal/day

| Day of Study | Subject | | | |
|---|---|---|---|---|
| | 0601 | 0603 | 0604 | 0605 |
| 21 | | | 2038 | |
| 22 | 2427 | | 1944 | |
| 23 | 2609 | 1460 | 2013 | |
| 24 | 2411 | 1606 | 1945 | |
| 25 | 2442 | 1545 | 1901 | |
| 26 | ---- | 1556 | 2075 | 2812 |
| 27 | 2609 | 1620 | 2114 | 2733 |
| 28 | 2552 | 1365 | 1810 | ---- |
| 29 | 2474 | 1522 | 1549 | 2881 |
| 30 | 2148 | 1593 | 2075 | 2621 |
| 31 | 2310 | 1694 | 1732 | |
| 32 | ---- | 1581 | 1987 | |
| 33 | 2429 | 1929 | 1825 | |
| 34 | | 1413 | 2463 | |
| 35 | | 2006 | 1786 | |
| 36 | | | 1787 | |
| average → | 2441 | 1607 | 1940 | 2762 |

*Ketosis and Uric Acid Clearance*

Daily total ketone body excretion, expressed as acetone, is shown for the subjects individually in Figures 6 to 10. The urinary output reflected gradual adjustment to the changes of diet, rising after the introduction of ketogenic regimes to achieve maximal concentration only by the third day or later. The rate of fall after switching from one of these diets to another, less ketogenic, one was also gradual. The data given in Table 13 are the averages of the two last days of each treatment period when values were most stable. These data show that the maximum renal output of ketones occurred with the 400 kcal fat diet, an average of 8 g per 24 hours for all subjects. The behavior of 0604, the diabetic subject, was erratic in this, her first period of study, in that she was resistant to ketosis from fat. The lowest average excretion of ketone was associated with carbohydrate-containing diets for all subjects. At 400 kcal intake from egg albumen, ketone formation was intermediate between that from fat and carbohydrate. Addition of 400 kcal as fat in the presence of 400 kcal of protein did not enhance ketosis, and addition of 400 kcal as carbohydrate prevented the ketosis found with protein alone.

Total blood ketones, measured as mg of acetone per 100 ml whole blood at the end of each dietary period, are plotted with the urinary data in Figures 6-10 and are collated in Table 14. These values are in good agreement with the urinary excretion patterns. Highest blood levels were found after feeding fat, in all except 0604, and ranged among the others from 19 to 25 mg%. Values were lowest with diets containing carbohydrate and in the two subjects for whom there are data, 200 kcal as carbohydrate was nearly as effective as was 400 kcal in diminishing ketogenesis, with the remaining 200 kcal from protein.

Only one subject was able to complete the glycine test. Her urinary ketone excretion was the same as that she experienced with an all-protein diet but the fasting blood value was nearly as low as that found after an overnight fast following the carbohydrate period. Her blood level with carbohydrate was the highest of the entire group of women, however.

Uric acid content of the urine is given in Table 15 and blood levels are recorded in Table 16. In general, these data correspond to the ketone values, blood levels directly and urinary clearances indirectly. That is, when ketonuria was maximal, urinary uric acid excretion was low and, under these conditions, blood levels of uric acid were elevated. In most subjects, this combination of events occurred with the pure fat diet; again, 0604 was exceptional in having less response to fat than to protein. Blood levels were lowest and urinary output highest with

## Figure 6. Daily Total Ketone Body Excretion and Periodic Blood Ketone Levels Subject 0601

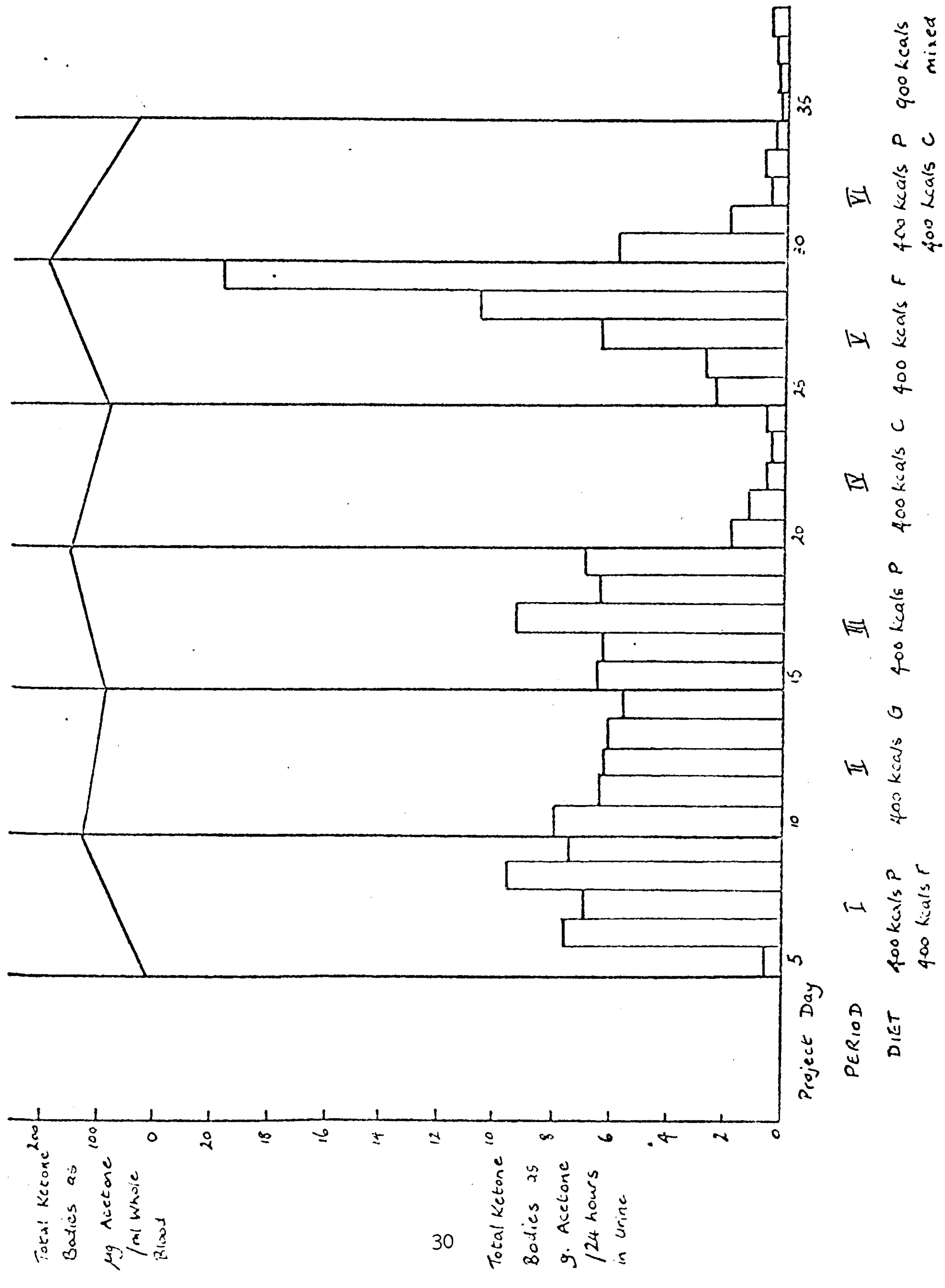


**Figure 7. Daily Total Ketone Body Excretion and Periodic Blood Ketone Levels Subject 0602**

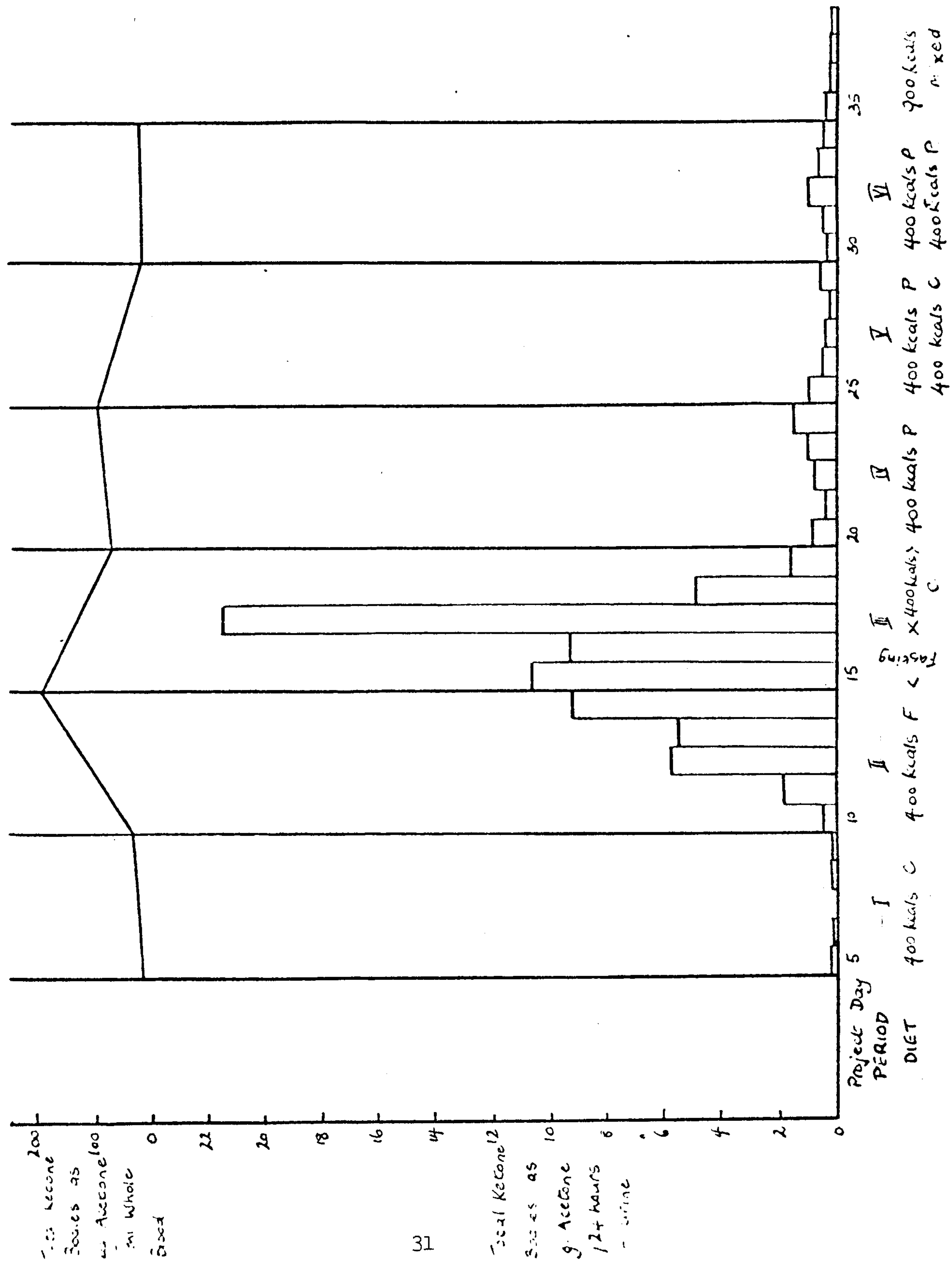


Figure 8. Daily Total Ketone Body Excretion and Periodic Blood Ketone Levels Subject 0603

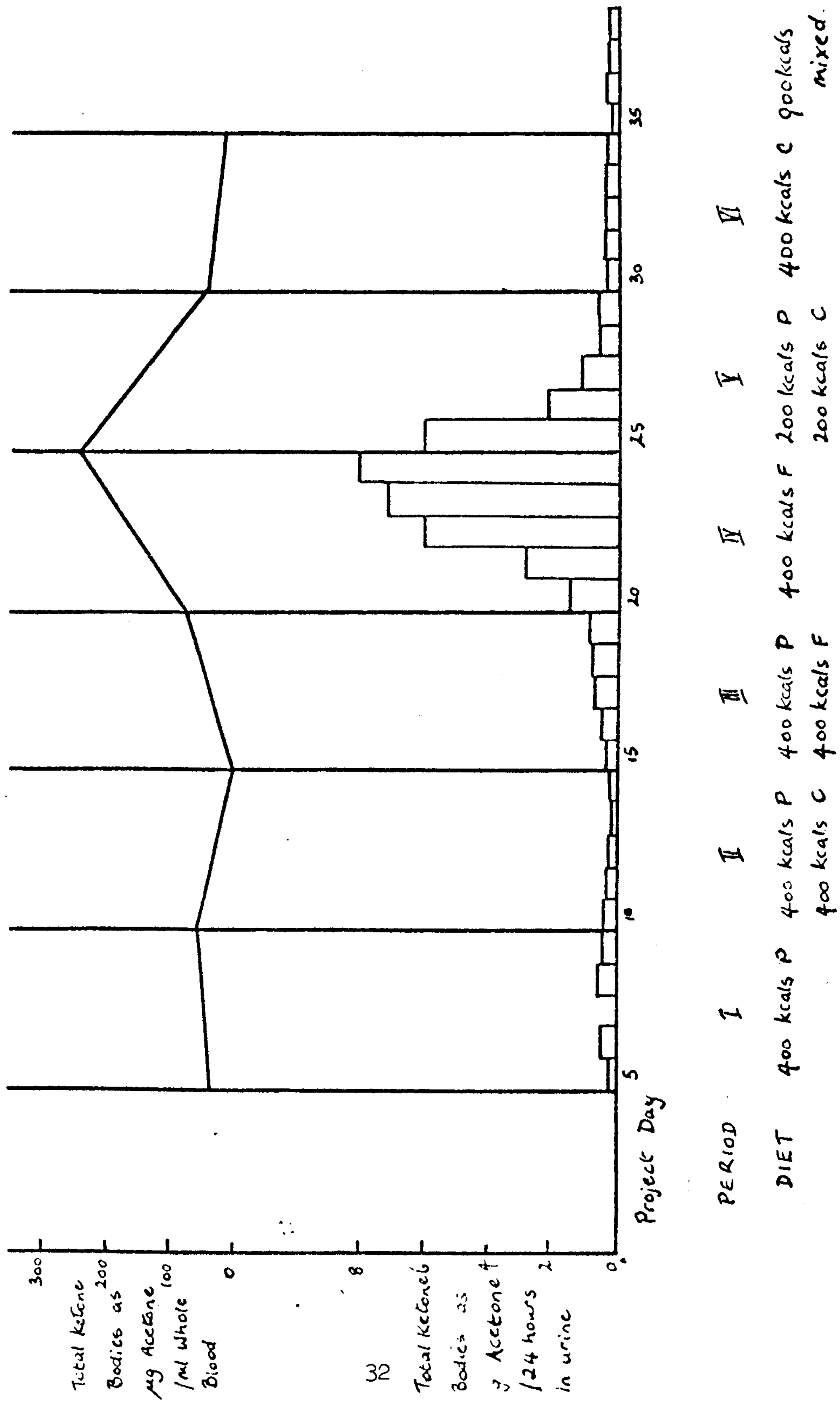
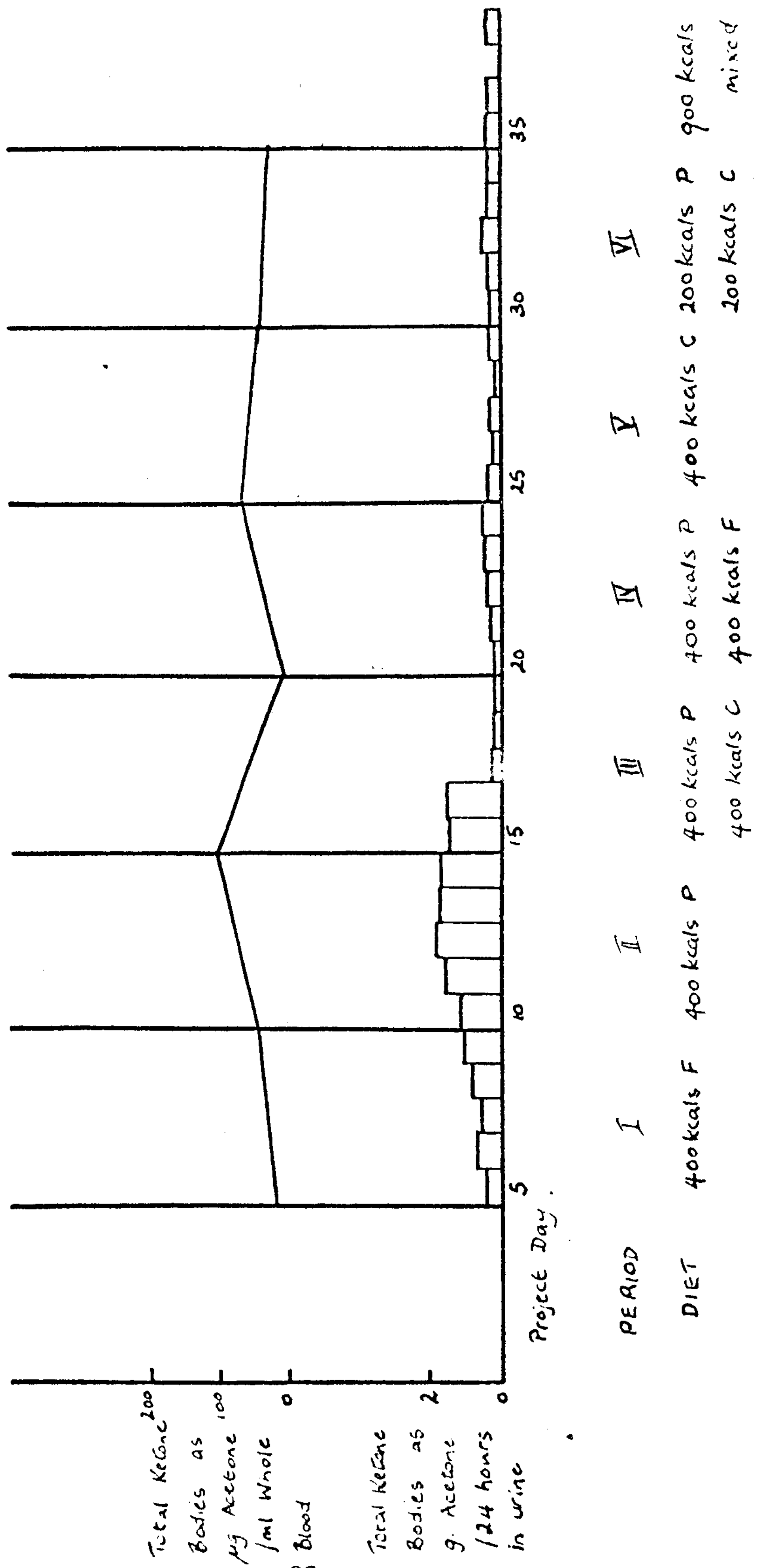


Figure 9. Daily Total Ketone Body Excretion and Periodic Blood Ketone Levels Subject c604



## Figure 10 Daily Total Ketone Body Excretion and Periodic Blood Ketone Levels Subject 0605

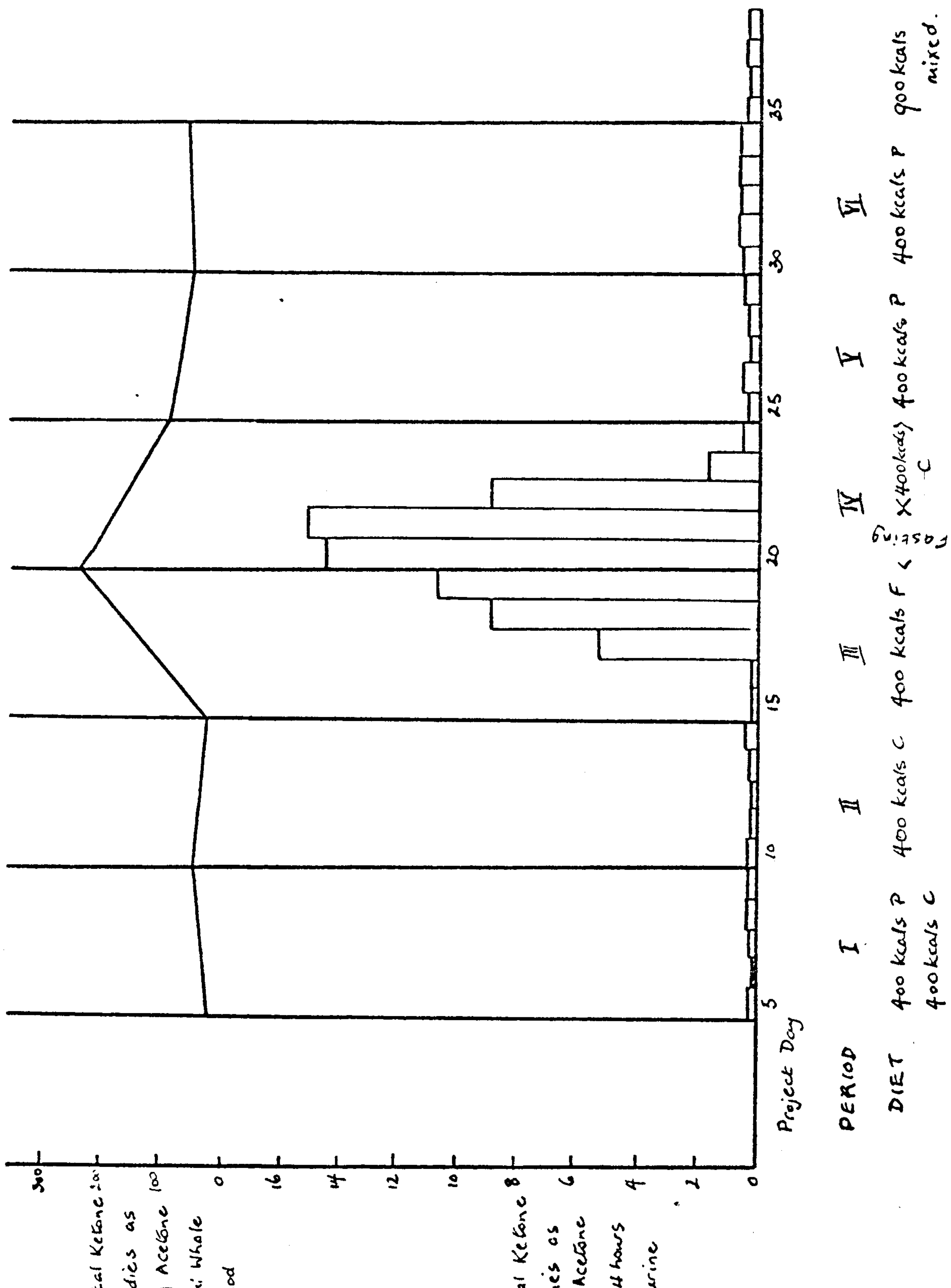


Table 13

URINARY KETONE DATA

g acetone/24 hrs., last 2 days each period

A. By period of study

| Subject | I | II | III | IV | V | VI | Post |
|---|---|---|---|---|---|---|---|
| 0601 | 7.844 | 5.795 | 6.572 | .378 | 15.068 | .437 | .320 |
| 0602 | .100 | 7.334 | 3.184 | 1.168 | .348 | .418 | .084 |
| 0603 | .410 | .104 | .708 | 7.556 | .563 | .322 | .280 |
| 0604 | .945 | 1.682 | .152 | .436 | .177 | .334 | .311 |
| 0605 | .194 | .196 | 9.713 | .933 | .226 | .528 | .288 |
| average → | 1.899 | 3.022 | 4.066 | 2.094 | 3.276 | .408 | .257 |

B. By dietary treatment

| | 400 kcal | | | | | 800 kcal | | | 900 kcal |
|---|---|---|---|---|---|---|---|---|---|
| | C | F | P | G | ½(C+P) | C+P | F+P | 2P | Mixed |
| 0601 | .378 | 15.068 | 6.572 | 5.795 | | .437 | 7.844 | | .320 |
| 0602 | .100 | 7.334 | 1.168 | (1) | | .348 | | .418 | .084 |
| 0603 | .322 | 7.556 | .410 | | .563 | .104 | .708 | | .280 |
| 0604 | .177 | .945 | 1.682 | | .334 | .152 | .436 | | .311 |
| 0605 | .196 | 9.713 | .528(3) | (2) | | .194 | | | .288 |
| average → | .235 | 8.123 | 2.072 | | .448 | .247 | 2.996 | | .257 |

(1) First 2 days essentially fasting + 3 days C: 3.184 g
(2) First 2 days essentially fasting + 3 days C; no minerals throughout period: .933 g
(3) Five days after no-mineral period: .226 g

Table 14

TOTAL BLOOD KETONE LEVELS AT END OF EACH DIETARY PERIOD

mg acetone/100 ml whole blood

| kcal/day → | 400 | | | | | 800 | | | 2000 |
|---|---|---|---|---|---|---|---|---|---|
| Diet → | C | F | P | G | C+P | C+P | F+P | P | Mixed |
| Subject | | | | | | | | | |
| 0601 | 8.45 | 18.83 | 15.15 | 8.92 | | 3.56 | 12.49 | | 1.17 |
| 0602 | 3.52 | 19.88 | 9.58 | (1) | | 1.30 | | 2.14 | 1.89 |
| 0603 | 1.87 | 24.66 | 5.92 | | 4.29 | 0.49 | 7.04 | | 3.38 |
| 0604 | 4.10 | 4.50 | 10.41 | | 2.31 | 1.57 | 6.79 | | 2.00 |
| 0605 | 2.08 | 23.42 | 5.48(3) | (2) | | 4.41 | | | 2.15 |
| average → | 4.00 | 18.26 | 9.31 | | | 2.27 | 8.77 | | 2.12 |

(1) First 2 days essentially fasting + 3 days 400 kcal C: 7.15 mg
(2) First 2 days essentially fasting + 3 days 400 kcal C; no minerals throughout period: 7.59 mg
(3) Days 6-10 of a 10-day period; 5 days after no-mineral period: 4.36 mg

Table 15

URIC ACID EXCRETION IN DIETARY PERIODS

mg/day

| kcal/day → | 400 | | | | | 800 | | |
|---|---|---|---|---|---|---|---|---|
| Diet → | C | F | P | G | C+P | C+P | F+P | P |
| **Subject** | | | | | | | | |
| 0601 | 292 | 146 | 163 | 378 | | 367 | 337 | |
| 0602 | 419 | 290 | 402 | (1) | | 475 | | 359 |
| 0603 | 385 | 174 | 267 | | 346 | 421 | 291 | |
| 0604 | 390 | 578 | 272 | | 412 | 296 | 360 | |
| 0605 | 364 | 223 | 378 (3) | (2) | | 287 | | |
| average → | 370 | 282 | 296 | | 379 | 369 | 329 | |

(1) First 2 days essentially fasting + 3 days C: 435 mg
(2) First 2 days essentially fasting + 3 days C; no minerals throughout period: 489 mg
(3) First 5-day period following period without minerals: 475 mg

Table 16

BLOOD URIC ACID LEVELS AT END OF EACH DIETARY PERIOD

mg/100 ml blood

| kcal/day → | 400 | | | | | 800 | | | 2000 |
|---|---|---|---|---|---|---|---|---|---|
| Diet → | C | F | P | G | C+P | C+P | F+P | P | Mixed |
| Subject | | | | | | | | | |
| 0601 | 6.5 | 9.4 | 8.6 | 5.7 | | 4.9 | 8.9 | | 5.2 |
| 0602 | 6.3 | 9.4 | 6.1 | (1) | | 4.0 | | 4.7 | 5.2 |
| 0603 | 5.1 | 9.1 | 7.6 | | 6.7 | 6.4 | 7.5 | | 5.4 |
| 0604 | 3.7 | 5.9 | 7.9 | | 4.1 | 3.5 | 4.2 | | 3.1 |
| 0605 | 5.3 | 8.9 | 5.6 (3) | (2) | | 4.8 | | | 4.1 |
| average → | 5.4 | 8.5 | 7.2 | | 5.4 | 4.7 | 6.9 | | 4.6 |

(1) First 2 days essentially fasting + 3 days C: 5.9 mg
(2) First 2 days essentially fasting + 3 days C; no minerals throughout period: 6.8 mg
(3) First 5-day period following period without minerals: 4.5 mg

diets containing carbohydrate, at any level of total caloric intake. For most of the subjects, urinary uric acid output was greater with protein than with fat, but lower than with carbohydrate. Blood levels reflected individual excretion patterns. These data indicate that blood uric acid levels are elevated with fat feeding as a result of failure adequately to clear uric acid through the kidney, and that in some subjects the same is true with a very low calorie, egg albumen diet. With glycine, urinary uric acid output was normal for the subject and blood concentration was not raised, suggesting that this amino acid can serve a similar function to dietary carbohydrate.

### *Excretion and External Balances*

Voluntary fluid intake varied from averages of 485 to 839 ml per day among the major dietary treatment periods (Table 17). The protein formula provided a larger amount of preformed dietary water than did the fat and carbohydrate mixtures, so even though self-administered water intake was lower in the 400 kcal protein period than with the non-protein diets, total water intake of the protein group was about 300 ml higher (3135 vs. 2849 and 2806 ml). Urinary volume (2072 g) and content of dissolved solids (440 mOsm per liter) were the same during the fat and protein 400 kcal periods, and less during consumption of carbohydrate (1553 g and 363 mOsm). The difference between fluid intake and urinary output is thus about 500 ml greater with the carbohydrate regimen than with the fat, and about 200 ml greater than with protein, which is still about 300 ml in excess of that with fat. Since there is no reason to suspect excessive loss of water as sweat or insensibly, and there were no digestive upsets, it is reasonable to conclude that these differences in crude balance indicate retention or loss of body water in response to diet.

At the 800 kcal level of intake, urinary volume was nearly the same for both diets (2300 g) but osmolality was higher with fat in the mixture than with carbohydrate (437 vs. 349 mOsm). Both dietary preformed and voluntary fluid intake were lower in the case of the fat-protein mixture, summating to a total difference of about 300 ml fluid intake. Therefore, the difference between intake and urinary volume was again larger in the presence of carbohydrate as at the lower caloric intakes. These differences in fluid correspond to the observed body weight responses of the subjects (vide supra), because weight loss was low with carbohydrate in the diet and high when fat was included.

Urinary pH was quite low throughout the experiment and did not vary markedly among dietary treatment periods. Urine was slightly less acid during the 400 kcal

Table 17

FLUID INTAKE AND
URINARY VOLUME, CONCENTRATION, AND pH

| Diet → | 400 kcal | | | | | 800 kcal | | |
|---|---|---|---|---|---|---|---|---|
| Subject | C | F | P | G | ½(C+P) | C+P | F+P | 2P |
| | Voluntary water intake, ml/day: | | | | | | | |
| 0601 | 792 | 820 | 463 | 625 | | 800 | 479 | |
| 0602 | 826 | 872 | 916 | | | 775 | | 587 |
| 0603 | 565 | 670 | 485 | | 480 | 540 | 430 | |
| 0604 | 958 | 980 | 665† | | 600 | 491 | 546 | |
| 0605 | 1020 | 855 | 812 | | | 970 | | |
| average → | 832 | 839 | 668 | | 540 | 715 | 485 | |
| | Pre-formed water from the diet, ml/day: | | | | | | | |
| | 2017 | 1967 | 2467 | 2717 | 2192 | 3017 | 2967 | 3467 |
| | Total average intake: | | | | | | | |
| | 2849 | 2806 | 3135 | | 2732 | 3732 | 3452 | |
| | Urinary volume, g/day | | | | | | | |
| 0601 | 1169 | 2554* | 2117 | 2109 | | 1720 | 2421 | |
| 0602 | 1780 | 1791 | 1324 | * | | 2932 | | 2796 |
| 0603 | 1881 | 1296 | 2152 | | 1701 | 2339 | 2763 | |
| 0604 | 1469 | 2554 | 2453† | | 2070 | 2210 | 1922 | |
| 0605 | 1466 | 2172 | 2313 | * | | 2118 | | |
| average → | 1553 | 2073 | 2072 | | 1886 | 2264 | 2369 | |
| | Urinary concentration, mosm/liter | | | | | | | |
| 0601 | 475 | 368 | 586 | 745 | | 328 | 504 | |
| 0602 | 378 | 457 | 430 | | | 298 | | 406 |
| 0603 | 256 | 684 | 471 | | 368 | 379 | 376 | |
| 0604 | 327 | 298 | 390† | | 298 | 352 | 431 | |
| 0605 | 380 | 395 | 325 | | | 389 | | |
| average → | 363 | 440 | 440 | | 333 | 349 | 437 | |
| | Urinary pH | | | | | | | |
| 0601 | 5.38 | 5.36 | 5.12 | 6.10 | | 5.79 | 5.35 | |
| 0602 | 5.82 | 5.03 | 5.56 | (1) | | 5.05 | | 4.87 |
| 0603 | 6.16 | 5.16 | 5.22 | | 5.15 | 5.00 | 4.91 | |
| 0604 | 5.18 | 5.40 | 4.72(3) | | 5.78 | 5.03 | 5.21 | |
| 0605 | 5.01 | 5.12 | 4.95 | (2) | | 4.94 | | |
| average → | 5.51 | 5.21 | 5.11 | | 5.46 | 5.16 | 5.15 | |

(1) First 2 days essentially fasting + 3 days C: 5.78
(2) First 2 days essentially fasting + 3 days C; no minerals throughout period: 5.78
(3) First 5-day period following period without minerals: 5.71
* Eresis † First protein period: intake, 958 ml; volume, 1626 g; concentration, 304 mosm

carbohydrate period than when protein and fat served as the sole sources of calories, but the mean value represents the effect of only two subjects in whom this was true. For the urinary pH to have remained nearly constant in the face of varying amounts of acidic ketone bodies and acidic end-products of protein metabolism in the urine (vide infra), basic elements and ammonia had to have been variably higher in the urine during some of the dietary periods.

Excretion patterns of two of the dominant basic elements of the urine, sodium and potassium, are shown in Table 18. Urinary content of both sodium and potassium was much greater when fat was present than when the diet contained carbohydrate at either level of caloric intake. The diet provided three grams of sodium per day; with fat-containing diets urinary excretion exceeded this amount. In three of the five subjects, urinary sodium also exceeded dietary intake during the all-protein period. This extra sodium would have been derived from the extracellular fluid (ECF) compartment. Usually there is a compensatory loss of ECF volume to maintain the compartment isoosmotic with the intracellular fluid (ICF). Only when sodium balance was positive, in the carbohydrate periods, was there an opportunity for retention of water with anomalous body weight behavior as a consequence, in which adipose tissue may have been lost with little or no apparent change in weight. If fluid was lost from the extracellular compartment, weight loss could have been spuriously high, in terms of true loss of adipose tissue, as with fat feeding.

The question of potassium is somewhat more complicated. Dietary intake was three grams per day, and with only one dietary treatment did the group average exceed this amount; when fat was given alone. In all other periods, urinary potassium was less than intake but balance cannot be assumed because, in contrast to sodium, there is significant fecal excretion of this element, normally, and fecal levels were not determined in this study. Since most subjects lost body nitrogen throughout the experiment (v.i.), and potassium is released when lean body tissue is catabolized, negative potassium balance would be anticipated. However, the isoosmotic relationships of the intra- and extracellular body fluid compartments can also be maintained by expansion of the intracellular fluid (diluting potassium, the dominant cation) to accommodate for loss of the extracellular cation, sodium, without shrinkage of the extracellular fluid volume. That is, the two compartments again reach equilibrium, but at a lower osmolarity. If this adjustment mechanism were used--and it well might have been to prevent dangerous shrinkage of the ECF, specifically the intravascular volume--potassium could actually have been retained in an expanded ICF space. Again, body weight relationships would have been distorted.

Table 18

URINARY SODIUM AND POTASSIUM

g/day

| Diet → Subject | 400 kcal C | 400 kcal F | 400 kcal P | 400 kcal G | 400 kcal ½(C+P) | 800 kcal C+P | 800 kcal F+P | 800 kcal 2P |
|---|---|---|---|---|---|---|---|---|
| | | | Sodium (Intake = 3.0 g) | | | | | |
| 0601 | 1.73 | 4.82 | 3.59 | 2.25 | | .67 | 3.88 | |
| 0602 | 2.91 | 3.46 | 1.74 | (1) | | 3.80 | | 2.86 |
| 0603 | 2.95 | 2.77 | 3.69 | | 2.13 | 2.55 | 4.32 | |
| 0604 | 2.48 | 3.14 | 3.35 | | 3.50 | 2.02 | 3.40 | |
| 0605 | 2.72 | 3.95 | 2.70 (3) | (2) | | 2.94 | | |
| average → | 2.56 | 3.63 | 3.01 | | 2.82 | 2.40 | 3.90 | |
| | | | Potassium (Intake = 3.0 g) | | | | | |
| 0601 | 1.73 | 4.09 | 2.55 | 2.78 | | 1.09 | 3.46 | |
| 0602 | 3.08 | 3.26 | 1.93 | (1) | | 2.15 | | 3.81 |
| 0603 | 2.60 | 3.04 | 3.01 | | 2.05 | 2.34 | 2.55 | |
| 0604 | 2.00 | 3.26 | 3.40 | | 2.62 | 2.05 | 1.91 | |
| 0605 | 2.04 | 3.77 | 2.20 (3) | (2) | | 2.72 | | |
| average → | 2.29 | 3.48 | 2.62 | | 2.33 | 2.07 | 2.64 | |

(1) First 2 days essentially fasting + 3 days C: sodium, 1.72; potassium, 2.18
(2) First 2 days essentially fasting + 3 days C; no minerals throughout period: sodium, 1.22; potassium, 1.86
(3) First 5-day period following period without minerals: sodium, .35; potassium, .40

Nitrogen excretion and balance data are listed in Table 19. Fecal nitrogen was increased when the diet contained protein, even though egg albumen is usually regarded as a nearly completely digestible protein and has been found to be when fed to our normal subjects in adequate diets. Fecal nitrogen was increased above the amount present in the feces when the diet contained no protein, by about 1.4 grams per day when the diet contained 12.87 grams of nitrogen, accounting for approximately 12% of the intake. Frequency of defecation and stool wet weight were also increased during protein feeding (Table 20).

Urinary excretion of nitrogen was higher with diets containing fat than with carbohydrate and higher in the presence of protein than in its absence. At the 400 kcal level, the sum of urinary and fecal nitrogen was less than intake (i.e., positive balance) in only one subject, given protein. In three of the remaining four subjects, nitrogen balance was no less negative when the diet included protein than it was when the same amount of non-protein calories was given. Most of the dietary protein was apparently used as an energy source, at the very low level of energy intake, and did little to spare body protein. When the caloric content of the diet was raised to 800 kcal with carbohydrate, two of the subjects achieved positive nitrogen balance and in two others the deficit was smaller than when no supplementary source of calories was given. However, the addition of fat calories did not improve nitrogen balance; in fact, the combination of fat and protein at 800 kcal was associated with the same destruction of tissue protein as occurred at 400 kcal without protein in the diet. In this regard, the addition of exogenous fat may be looked upon as no improvement over allowing the subject to use her own endogenous fat to support her energy needs.

True nitrogen balance was not measured in this study, because we have no information on dermal nitrogen loss and integumentary growth in these women. We did attempt to estimate menstrual nitrogen losses by extracting the sanitary napkins used during each period but the values were unreliable. In spite of precautionary measures, the urine was occasionally visibly contaminated at this time. The test periods in which menses occurred are designated in the table; 0601 did not menstruate during the 30 days of collection and 0604 was post-menopausal. Blood samples taken during the study accounted for an additional nitrogen loss of about 140 mg per subject per day. Thus, loss of tissue protein is underestimated significantly.

Urinary creatinine output was not truly constant as is often assumed. These data, as well as those for other measured parameters, were investigated for period

Table 19

NITROGEN BALANCE

g/day

| Diet → | 400 kcal C | 400 kcal F | 400 kcal P | 400 kcal G | 400 kcal ½(C+P) | 800 kcal C+P | 800 kcal F+P | 800 kcal 2P | |
|---|---|---|---|---|---|---|---|---|---|
| Nitrogen Intake → | 0.20 | 0.20 | 12.87 | 20.06 | 6.44 | 12.87 | 12.87 | 25.45 | |
| **Subject** | | | | Fecal nitrogen | | | | | **Average** |
| 0601 | .65 | .70 | .13 | 1.27 | | .65 | .62 | | .77 |
| 0602 | 1.05 | .81 | 5.10 | (1) | | 1.82 | | 2.41 | 2.17 |
| 0603 | .90 | 1.06 | 1.90 | | 1.56 | 4.22 | 3.98 | | 2.21 |
| 0604 | 1.03 | .83 | 3.54 (3) | | .79 | 1.31 | 2.74 | | 1.85 |
| 0605 | 1.09 | .24 | .52 (3) | (2) | | 2.04 | | | 1.11 |
| average → | .94 | .73 | 2.24 | 1.27 | 1.18 | 2.01 | 2.47 | 2.41 | |
| | | | | Urinary nitrogen | | | | | |
| 0601 | 4.19 | 5.09 | 16.11 | 25.31 | | 8.24 | 17.40 | | |
| 0602 | 7.32 | 7.53 | 9.48 | (1) | | 12.24 | ----- | 15.13 | |
| 0603 | 3.50 | 8.32 | 15.74 | | 7.94 | 13.28 | 19.55 | | |
| 0604 | 4.77 | 8.78 | 11.83 (3) | | 6.44 | 11.15 | 11.64 | | |
| 0605 | 5.61 | 6.29 | 10.57 (3) | (2) | | 14.25 | | | |
| average → | 5.08 | 7.20 | 12.75 | | 7.19 | 11.83 | 16.20 | | |
| | | | | Nitrogen balance [Intake - (Urinary + Fecal)] | | | | | |
| 0601 | -4.64 | -5.59 | -3.37 | -6.52 | | +3.99 | -5.15 | | |
| 0602 | -8.68* | -8.24* | -1.81 | (1) | | -1.29 | | +7.91 | |
| 0603 | -4.20 | -9.18 | -4.77* | | -3.06 | -4.63 | -10.66 | | |
| 0604 | -5.60 | -9.41 | -2.50 (3) | | -0.46 | +0.41 | -1.51 | | |
| 0605 | -6.50* | -6.33 | +1.78 (3) | (2) | | -3.42* | | | |
| average → | -5.92 | -7.75 | -2.13 | | -1.76 | -0.99 | -5.77 | | |

(1) First 2 days essentially fasting + 3 days C: fecal, .52; urinary, 13.41; balance, -13.60

(2) First 2 days essentially fasting + 3 days C; no minerals throughout period: fecal, .68; urinary, 6.86; balance, -7.42

(3) First 5-day period following period without minerals: fecal, 1.11; urinary, 8.17; balance, -13.59 *Menses

Table 20

STOOL FREQUENCY AND WET WEIGHT

| Diet → | 400 kcal | | | | | 800 kcal | | |
|---|---|---|---|---|---|---|---|---|
| Subject | C | F | P | G | ½(C+P) | C+P | F+P | 2P |
| | Bowel movements, number per 5-day period | | | | | | | |
| 0601 | 2 | 3 | 1 | 4 | | 4 | 2 | |
| 0602 | 7 | 10 | 16 | (1) | | 3 | | 5 |
| 0603 | 3 | 3 | 5 | | 3 | 6 | 6 | |
| 0604 | 6 | 5 | 9 | | 4 | 4 | 9 | |
| 0605 | 5 | 2 | 3(3) | (2) | | 5 | | |
| average → | 4.6 | 4.6 | 6.8 | | 1.6 | 4.4 | 5.7 | |
| | Wet weight of feces, g/day | | | | | | | |
| 0601 | 74 | 87 | 20 | 155 | | 87 | 103 | |
| 0602 | 181 | 247 | 402 | (1) | | 136 | | 154 |
| 0603 | 112 | 116 | 112 | | 103 | 211 | 241 | |
| 0604 | 117 | 147 | 205 | | 90 | 111 | 236 | |
| 0605 | 116 | 24 | 41(3) | (2) | | 151 | | |
| average → | 120 | 124 | 156 | | 96 | 139 | 193 | |

(1) First 2 days essentially fasting + 3 days C: number = 3; weight = 106 g
(2) First 2 days essentially fasting + 3 days C; no minerals throughout period: number = 3; weight = 68 g
(3) First protein period following period without minerals; number = 3; weight = 56

effects in addition to major dietary treatment effects. Creatinine excretion was the only measure, in addition to body weight change, that showed a systematic change with time, in this case a decrease. Creatinine output was not related to dietary composition (Table 21). (There appeared also to have been a decrease in the rate of body nitrogen loss, as reflected in nitrogen balance, as the study progressed. Considering the change in lean body mass with tissue loss throughout the study, some diminution in output would be anticipated if the catabolism per unit mass remained even constant. In addtion, the data are somewhat confounded because there was a preponderance of protein-containing diets in the last period of the experiment, occasioned by the subjects' refusal to accept the programmed diets.)

## *Internal Balances*

Estimates of changes in body composition may be computed from the rough balance data available. These computations indicate that loss of fat-free protoplasm (nitrogen balance in grams x 29.2, Ref. 12) was greatest during the period of 400 kcal-fat diet, with a group mean of 1132 grams per five-day period. Loss was somewhat less when the fat diet was supplemented with an additional 400 kcal of protein (843 g). The same amount of protoplasm was catabolized when 400 kcal of carbohydrate was given (865 g) and only 312 g when the diet was egg albumen alone (Table 22). Tissue protein loss was widely variable when the diet contained both protein and carbohydrate, at the 800 kcal level, ranging from an apparent gain of over 500 grams per period (0601) to a loss of nearly 700 g (0603).

Alteration in extracellular fluid can be computed from sodium balance, assuming that the concentration of sodium in the ECF is unchanged. (As we have discussed above, this assumption is not always justified.) For this calculation, fecal sodium may be taken to be about 1 mEq daily. ECF may then be derived from sodium balance in grams x 287.94 less nitrogen balance in grams x 5, Ref. 12). On this basis, loss of ECF was greatest with the 800 kcal diet containing fat plus protein (1168 g per 5-day period) and less with the 400 kcal-fat treatment alone (742 g) (Table 23). When carbohydrate was given, gains of ECF were indicated, amounting to about 800 g per period, irrespective of caloric allowance.

Computations of intracellular fluid are less well grounded, because fecal loss is unknown and can be variable. If an average fecal value of 120 mg per day is accepted, then potassium balance is positive in all cases in which the diet contained carbohydrate, and expansion of ICF is indicated (Table 23). About half of the cases of feeding fat, protein, or fat plus protein showed apparent negative balances of this element, suggesting loss of ICF.

Table 21

URINARY CREATININE

g/day

A. By period of study

| Subject | I | II | III | IV | V | VI |
|---|---|---|---|---|---|---|
| 0601 | 1.54 | 1.36 | 1.44 | 1.14 | 1.34 | 1.19 |
| 0602 | 1.70 | 1.71 | 1.66 | 1.56 | 1.65 | 1.55 |
| 0603 | 1.36 | 1.44 | 1.42 | 1.39 | 1.31 | 1.26 |
| 0604 | 1.55 | 1.49 | 1.47 | 1.40 | 1.28 | 1.33 |
| 0605 | 1.30 | 1.24 | 1.28 | 1.25 | 1.17 | 1.14 |
| average → | 1.49 | 1.45 | 1.45 | 1.35 | 1.35 | 1.29 |

B. By dietary treatment

| | 400 kcal | | | | | 800 kcal | | |
|---|---|---|---|---|---|---|---|---|
| | C | F | P | G | ½(C+P) | C+P | F+P | 2P |
| 0601 | 1.14 | 1.34 | 1.44 | 1.36 | | 1.19 | 1.54 | |
| 0602 | 1.70 | 1.71 | 1.56 | (1) | | 1.56 | | 1.55 |
| 0603 | 1.26 | 1.39 | 1.36 | | 1.31 | 1.44 | 1.42 | |
| 0604 | 1.28 | 1.55 | 1.49 | | 1.33 | 1.47 | 1.40 | |
| 0605 | 1.24 | 1.28 | 1.14(3) | (2) | | 1.30 | | |
| average → | 1.32 | 1.45 | 1.40 | | 1.32 | 1.41 | 1.45 | |

(1) First 2 days essentially fasting + 3 days C: 1.66 g
(2) First 2 days essentially fasting + 3 days C; no minerals throughout period: 1.25 g
(3) First 5-day period following period without minerals: 1.17 g

Table 22

ESTIMATED CHANGE IN FAT-FREE PROTOPLASM

g/day based on nitrogen balance

A. By period of study

| Subject | I | II | III | IV | V | VI |
|---|---|---|---|---|---|---|
| 0601 | - 751.9 | - 951.9 | - 492.0 | - 677.4 | - 816.1 | + 581.1 |
| 0602 | -1267.3 | -1203.0 | -1985.6 | - 264.3 | - 188.3 | +1154.9 |
| 0603 | - 696.4 | - 676.0 | -1556.4 | -1340.3 | - 446.8 | - 613.2 |
| 0604 | -1373.9 | - 365.0 | + 59.9 | - 220.5 | - 817.6 | - 67.2 |
| 0605 | - 499.3 | - 949.0 | - 924.2 | -1083.3 | + 524.1 | + 259.9 |
| average → | - 917.8 | - 829.0 | - 979.7 | - 717.2 | - 348.9 | + 263.1 |

B. By dietary treatment

| | 400 kcal | | | | | 800 kcal | | |
|---|---|---|---|---|---|---|---|---|
| | C | F | P | G | ½(C+P) | C+P | F+P | 2P |
| 0601 | - 677.4 | - 816.1 | - 492.0 | - 951.9 | | + 581.1 | - 751.9 | |
| 0602 | -1267.3 | -1203.0 | - 264.3 | (1) | | - 188.3 | | +1154.9 |
| 0603 | - 613.2 | -1340.3 | - 696.4 | | - 446.8 | - 676.0 | -1556.4 | |
| 0604 | - 817.6 | -1373.9 | - 365.0 | | - 67.2 | + 59.9 | - 220.5 | |
| 0605 | - 949.0 | - 924.2 | + 259.9 (3) | (2) | | - 499.3 | | |
| average → | - 864.9 | -1131.5 | - 311.6 | | - 257.0 | - 144.5 | - 842.9 | |

(1) First 2 days essentially fasting + 3 days C: -1985.6 g
(2) First 2 days essentially fasting + 3 days C; no minerals throughout period: -1083.3 g
(3) First 5-day period following period without minerals: +524.1 g

Table 23

CHANGE IN EXTRACELLULAR AND INTRACELLULAR FLUID

computed from urinary excretion of cations

| Kcal/day → | 400 | | | | | 800 | | |
|---|---|---|---|---|---|---|---|---|
| Diet / Subject | C | F | P | G | ½(C+P) | C+P | F+P | 2P |
| | Estimated change in extracellular fluid, based on Na balance* g/period | | | | | | | |
| 0601 | 1904.2 | -2521.4 | - 792.5 | 1187.0 | | 3214.9 | -1177.5 | |
| 0602 | 331.3 | - 465.5 | 1742.8 | (1) | | -1155.4 | | - 45.3 |
| 0603 | 161.4 | 491.0 | - 909.6 | | 1302.1 | 670.0 | -1721.4 | |
| 0604 | 866.0 | 16.0 | 526.9 | | - 725.1 | 1342.1 | - 603.6 | |
| 0605 | 523.0 | -1230.8 | 361.4 (3) | (2) | | 105.4 | | |
| average → | 757.2 | - 742.1 | 185.8 | | 115.4 | 835.4 | -1167.5 | |
| | Estimated change in intracellular fluid, based on K balance** g/period | | | | | | | |
| 0601 | 1483.0 | - 545.0 | 676.4 | 661.18 | | 1238.3 | - 16.12 | |
| 0602 | 751.5 | 571.1 | - 958.3 | (1) | | 714.9 | | -1518.0 |
| 0603 | 755.1 | 628.3 | 366.0 | | 1096.4 | 732.5 | 1140.2 | |
| 0604 | 1399.0 | 650.0 | - 389.6 | | 377.3 | 756.3 | 928.4 | |
| 0605 | 1372.7 | - 113.6 | 505.7 (3) | (2) | | 380.0 | | |
| average → | 1152.3 | 238.2 | 40.0 | | 736.8 | 764.4 | 684.2 | |

(1) First 2 days essentially fasting + 3 days C: Extracellular, 452.1; intracellular, 1982.5.
(2) First 2 days essentially fasting + 3 days C; no minerals throughout period: Extracellular, 986.2; intracellular, 1653.0.
(3) First 5-day period following period without minerals: Extracellular, 3684.8; intracellular, 1892.1.
* Fecal Na assumed to be .03 g/day **Fecal K assumed to be .12 g/day

Total changes for the study are listed in Table 24, together with several other measures of body composition. Circulating blood volume was found to be decreased in four of the five subjects, only 0601 showing a gain in this compartment which corresponds with her observed recovery of body weight in the terminal period of the experiment. Three of the four subjects who had measured losses of blood volume were predicted to have increases in ECF based on sodium balance. It is interesting that the circulating blood volumes of these obese women did not correspond to the usually accepted value of about 8% of body weight as blood, and would be appropriate to persons weighing only 70 kg. Adipose tissue does contain a significant vascular supply, so the net effect of this small intravascular fluid space must be a relatively poor blood supply to some of the body tissues. In spite of probable fluid shifts, there was no consistent difference in either resting blood pressure, or cardiovascular responsiveness to tilt during the study (Tables 25 and 26).

Only two of the subjects were willing to be weighed under water for determination of body density and these had to be weighed in the swimming pool as they could not be comfortably measured in our tank constructed for the purpose. Comparison of initial and final densities indicated relatively minor losses of lean body mass (0.9 and 1.8 kg) and major losses of adipose tissue (11.8 and 20.8 kg) (Table 24). Similar estimations from total body water determined by dilution of tritiated water gave widely disparate values. During the initial observation with tritiated body water the subjects experienced a diuresis, so that the use of a conventional factor to convert this to lean body mass and, from this, body fat is impossible.

None of these measures agrees well with the losses of protein tissue estimated from nitrogen balance data. These data suggest losses of fat-free protoplasm ranging from 2.8 to 4.9 kg for the study. Urinary creatinine is also assumed to bear a constant relationship to muscle tissue and so can be used to estimate muscle mass (Ref. 13). The decrease in creatinine excretion during the experiment is computed to reflect decreases of 2.1 to 5.3 kg of muscle tissue. These values are in the same range as predicted from nitrogen balance, but subjects do not show the same rank-ordering by the two methods of estimation.

Body dimensions were measured, largely as an incentive to the women, but did

Table 24

## MEASURES OF BODY COMPOSITION

| | 0601 | 0602 | 0603 | 0604 | 0605 |
|---|---|---|---|---|---|
| Circulating blood volume, dye dilution method, liters | | | | | |
| Initial | 4.77 | 5.54 | 6.19 | 7.01 | 5.39 |
| Final | 5.35 | 4.85 | 5.33 | 6.54 | 5.02 |
| Change | +.58 | -.69 | -.86 | -0.47 | -0.37 |
| Change in extracellular fluid space, calc. from sodium balance, kg | | | | | |
| | +1.8 | +0.9 | ess. 0 | +0.4 | +4.4 |
| Change in fat-free protoplasm, calc. from nitrogen balance, kg | | | | | |
| | -3.2 | -3.9 | -4.9 | -2.9 | -2.8 |
| Muscle tissue calc. from urinary creatinine, kg | | | | | |
| Initial | 29.2 | 34.0 | 27.2 | 31.1 | 26.2 |
| Final | 23.9 | 31.1 | 25.1 | 26.7 | 22.8 |
| Change | -5.3 | -2.9 | -2.1 | -4.4 | -3.4 |
| Lean body mass from total body water ($T_2O$), kg | | | | | |
| Initial | 74.5 | 75.0 | 79.3 | 75.6 | 72.6 |
| Final | 61.5 | 61.9 | 61.5 | 57.0 | 60.2 |
| Change | -13.0 | -13.1 | -17.8 | -18.6 | -12.4 |
| Body composition by densitometry (underwater weight), kg | | | | | |
| Lean body mass | | | | | |
| Initial | 59.0 | | | | 56.5 |
| Final | 57.2 | | | | 55.6 |
| Change | -1.8 | | | | -0.9 |
| Adipose tissue | | | | | |
| Initial | 64.2 | | | | 87.9 |
| Final | 52.4 | | | | 67.1 |
| Change | -11.8 | | | | -20.8 |

Table 25

RESTING BLOOD PRESSURE

mm Hg

| Diet → | 400 kcal | | | | | 800 kcal | | |
|---|---|---|---|---|---|---|---|---|
| Subject | C | F | P | G | ½(C+P) | C+P | F+P | 2P |
| 0601 | 102/64 | 120/72 | 118/72 | 108/58 | | 120/64 | 140/68 | |
| 0602 | 154/108 | 130/98 | 142/80 | (1) | | 156/90 | | 124/84 |
| 0603 | 136/84 | 164/78 | 162/86 | | 160/86 | 160/100 | 152/78 | |
| 0604 | 138/84 | 142/80 | 160/100 | (2) | 120/70 | 142/88 | 122/84 | |
| 0605 | 120/68 | 154/80 | 124/80 (3) | | | 104/80 | | |

(1) First 2 days essentially fasting + 3 days C: 152/92 mm Hg
(2) First 2 days essentially fasting + 3 days C; no minerals throughout period: 124/76
(3) First 5-day period following period without minerals: 122/88

Table 26

TILT TABLE RESPONSE

| Subject | Day of Study | 0° BP* | 0° P* | 0° R* | 30 sec. at 60° BP | 60 sec. at 90° BP | 60 sec. at 90° P | 60 sec. at 90° R |
|---|---|---|---|---|---|---|---|---|
| 0601 | 5 | 128/82 | 84 | 18 | 134/104 | 118/90 | 90 | |
| | 10 | 140/68 | 76 | 18 | 130/60 | 130/88 | | |
| | 15 | 108/58 | 70 | 16 | 108/60 | 110/60 | 76 | 18 |
| | 20 | 118/72 | 84 | 18 | 118/72 | 116/72 | 80 | 18 |
| | 25 | 102/64 | 76 | 18 | 102/66 | 102/70 | 84 | 18 |
| | 30 | 120/72 | 90 | 16 | 112/66 | 116/72 | 94 | 18 |
| | 35 | 120/60 | 88 | 18 | 120/64 | 122/68 | 90 | 20 |
| 0602 | 5 | 158/106 | 88 | 16 | 152/92 | 126/92 | | |
| | 10 | 154/108 | 72 | 18 | 130/98 | 140/90 | 72 | 18 |
| | 15 | 130/98 | 64 | 16 | 130/98 | 140/106 | 74 | 18 |
| | 20 | 152/92 | 70 | 18 | 148/90 | 148/94 | 90 | 15 |
| | 25 | 142/80 | 56 | 16 | 120/88 | 128/78 | 76 | 18 |
| | 30 | 156/90 | 60 | 16 | 136/94 | 130/92 | 80 | 18 |
| | 35 | 124/84 | 78 | 20 | 122/82 | 120/80 | 80 | 18 |
| 0603 | 5 | 142/78 | 84 | 18 | 138/88 | 148/82 | 96 | 18 |
| | 10 | 162/86 | 76 | 18 | 158/72 | 130/82 | | |
| | 15 | 160/100 | | | 156/100 | 160/108 | 88 | 22 |
| | 20 | 152/78 | 96 | 15 | 148/72 | 146/70 | 96 | 15 |
| | 25 | 164/78 | 88 | 16 | 124/88 | 130/84 | 102 | 22 |
| | 30 | 160/86 | 80 | 22 | 146/80 | 138/88 | 94 | 20 |
| | 35 | 136/84 | 88 | 22 | 140/88 | 140/90 | 100 | 24 |
| 0604 | 5 | 140/100 | 68 | 16 | 140/104 | 146/108 | | |
| | 10 | 142/80 | 88 | 18 | 140/100 | 132/100 | | |
| | 15 | 160/100 | 120 | 24 | 140/90 | 122/80 | 78 | 20 |
| | 20 | 142/88 | 72 | 18 | 140/84 | 138/84 | 84 | 18 |
| | 25 | 122/84 | 80 | 16 | 110/88 | 116/84 | 78 | 16 |
| | 30 | 138/84 | 74 | 16 | 118/78 | 108/80 | | |
| | 35 | 120/70 | 80 | 18 | 126/78 | 128/90 | 90 | 22 |
| 0605 | 5 | 124/58 | 88 | 18 | 128/88 | 148/88 | | |
| | 10 | 144/80 | 80 | 18 | 144/80 | 110/70 | | |
| | 15 | 120/68 | 72 | 18 | 124/70 | 130/72 | | |
| | 20 | 154/80 | 80 | 18 | 150/78 | | | |
| | 25 | 124/76 | 100 | 24 | 116/88 | 114/88 | 104 | 18 |
| | 30 | 122/88 | 74 | 18 | 134/76 | 124/76 | 76 | 16 |
| | 35 | 124/80 | 78 | 20 | 120/78 | 124/88 | 84 | 22 |

* BP= Blood Pressure in mm Hg ; P = heart rate, beats per min. ; R= breathing rate, respirations per min.

reveal interesting differences in loss at various body sites (Table 27). Most of the subjects weighed at the end of the sixth period about 91-92% as much as they did at the beginning of the first period. Circumference of the upper arm was only 85% of the initial measurement at the end of the study; and the thigh and neck were affected to a greater extent (92 and 94% of initial dimension) than were the chest, waist and hips (99% of initial). Mayer has found that the skin-fold thickness of the upper arm is more indicative of recent nutritional history (with regard to caloric insufficiency and excess) than are measures in other customary locations. Our data concur.

### *Other Blood Constituents*

Surprising variation in blood glucose levels was seen in response to administration of the several diets (Table 28). Fasting levels were measured on the morning after the fifth day of feeding a given diet. Blood sugar was higher when the carbohydrate diet was given than when the diet provided 400 kcal as fat, for all subjects. The same is true, comparing the pair of 800 kcal diets. For four of the women, blood sugar was higher with protein feeding than with an exclusively fat diet, but lower than with carbohydrate. The fifth, erratic subject, 0605, had a higher blood level with protein than with sugar. Blood glucose was low in the young subject, 0601, and during her 400 kcal fat period, it was near the hypoglycemic level. Values were consistently higher in the controlled, diabetic subject, 0604, but were well below her pre-test value.

Serum total cholesterol declined immediately upon initiation of the weight reduction program, from a pre-test level of 259 mg% to 196 mg% at the end of the 2000 kcal "standardization" period. An additional decrease occurred with introduction of the very low-calorie diets and levels were essentially constant thereafter, until the last period, when a small upswing was recorded. Dietary treatments were without effect.

Blood urea nitrogen reflected the amount of nitrogen fed, and no other factor.

## B. BRIEF STUDY OF NORMAL SUBJECTS

### *Urinary Nitrogen Excretion and Weight Loss*

Subjects of nearly normal weight excreted more nitrogen in the urine during consumption of the low calorie, all casein diet than did the obese women of experiment no. 6 fed only egg albumen (see Tables 19 and 29). Excretions were quite variable on the first test day and much lower than on subsequent days, probably due

Table 27

## FINAL BODY MEASUREMENTS
Dimensions and terminal values as percent of original

| Subject | | Neck | Upper Arm | Chest | Waist | Hips | Thigh | Calf | Weight** |
|---|---|---|---|---|---|---|---|---|---|
| 0601 | Initial* | 39 | 34 | 1017 | 1012 | 1035 | 66 | 44 | 120.09 |
| | Final* | 37 | 31 | 1009 | 1001 | 1019 | 60 | 42 | 108.2 |
| | Percent | 94 | 93 | 99 | 99 | 98 | 92 | 95 | 90.5 |
| 0602 | Initial | 39 | 42 | 1015 | 1014 | 1026 | 68 | 48 | 114.41 |
| | Final | 37 | 37 | 1006 | 1011 | 1024 | 65 | 47 | 103.71 |
| | Percent | 95 | 88 | 99 | 99 | 100 | 94 | 98 | 90.6 |
| 0603 | Initial | 42 | 46 | 1022 | 1020 | 1052 | 80 | 47 | 138.40 |
| | Final | 40 | 40 | 1014 | 1012 | 1035 | 73 | 47 | 127.54 |
| | Percent | 95 | 87 | 99 | 99 | 98 | 91 | 100 | 92.2 |
| 0604 | Initial | 40 | 46 | 1019 | 1022 | 1052 | 74 | 49 | 132.52 |
| | Final | 37 | 39 | 1009 | 1008 | 1026 | 66 | 49 | 125.13 |
| | Percent | 92 | 85 | 99 | 99 | 99 | 90 | 100 | 92.9 |
| 0605 | Initial | 36 | 43 | 1031 | 1017 | 1058 | 73 | 49 | 132.0 |
| | Final | 34 | 30 | 1007 | 98 | 1042 | 68 | 46 | 121.75 |
| | Percent | 92 | 70 | 98 | 97 | 98 | 94 | 93 | 92.2 |
| | Average Percent → | 93.6 | 84.6 | 98.9 | 98.6 | 98.9 | 92.1 | 97.3 | 91.7 |

* Body measurements given in cm; **Weight in kg

Table 28

BLOOD GLUCOSE, CHOLESTEROL AND UREA NITROGEN AFTER VARIOUS DIETARY TREATMENTS

| Subject | Before Study | Diet → 2000 kcal Mixed | 400 kcal C | 400 kcal F | 400 kcal P | 400 kcal G | 400 kcal ½(C+P) | 800 kcal C+P | 800 kcal F+P | 800 kcal 2P |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Blood glucose, mg/100 ml | | | | | | | |
| 0601 | 78 | 85 | 76 | 46 | 65 | 68 | | 92 | 52 | |
| 0602 | 98 | 109 | 97 | 77 | 84 | (1) | | 100 | | 108 |
| 0603 | 120 | 125 | 136 | 75 | 87 | | 90 | 105 | 77 | |
| 0604 | 252 | 252 | 155 | 138 | 119 (3) | | 170 | 118 | 102 | |
| 0605 | * | 109 | 86 | 56 | 109 (3) | (2) | | 88 | | |
| average → | | 136 | 110 | 78 | 93 | | 130 | 101 | 77 | |
| | | | Serum total cholesterol, mg/100 ml | | | | | | | |
| 0601 | 224 | 160 | 120 | 123 | 130 | 124 | | 145 | 146 | |
| 0602 | 292 | 235 | 212 | 190 | 187 | (1) | | 197 | | 244 |
| 0603 | 219 | 182 | 173 | 146 | 173 | | 148 | 165 | 160 | |
| 0604 | 302 | 255 | 186 | 246 | 212 (3) | | 219 | 226 | 207 | |
| 0605 | * | 150 | 128 | 140 | 144 (3) | (2) | | 129 | | |
| average → | 259 | 196 | 164 | 169 | 169 | | 184 | 172 | 171 | |
| | | | Blood urea nitrogen, mg/100 ml | | | | | | | |
| 0601 | 13 | 12.5 | 2.0 | 4.0 | 11.5 | 16.5 | | 11.0 | 12.5 | |
| 0602 | 13.0 | 15.0 | 6.5 | 6.5 | 13.5 | (1) | | 12.0 | | 22.0 |
| 0603 | 11.5 | 12.0 | 8.0 | 4.5 | 16.5 | | 8.5 | 14.5 | 15.5 | |
| 0604 | 16.5 | 14.0 | 6.0 | 6.5 | 16.0 (3) | 13. | 13.5 | 12.0 | 11.0 | |
| 0605 | * | 12.5 | 4.5 | 5.0 | 9.5 (3) | (2) | | 12.5 | | |
| average → | 13.4 | 13.2 | 5.4 | 5.3 | 13.4 | | 11.0 | 12.4 | 13.0 | |

(1) First 2 days essentially fasting + 3 days C: glucose, 96; cholesterol, 179; BUN, 3.5.
(2) First 2 days essentially fasting + 3 days C; no minerals throughout period: glucose, 103; cholesterol, 138; BUN, 2.5.
(3) First 5-day period following period without minerals: glucose, 93; cholesterol, 196; BUN, 13.
* Not fasting blood: glucose, 93; cholesterol, 196; BUN, 13.

Table 29

WEIGHT LOSS AND URINARY NITROGEN EXCRETION OF NORMAL SUBJECTS CONSUMING DAILY 95 g OF CASEIN* OR A PROTEIN-FREE DIET, g/day

| | Female Subjects | | | | Male Subjects | | |
|---|---|---|---|---|---|---|---|
| | Ba | C | G | N | Bo | M | |
| Nitrogen Intake, g/day → | 13.1 | 13.1 | 13.1 | 13.1 | 13.1 | 13±** | 0.** |
| Day of Treatment | | | | | | | |
| 1 | 15.13 | 12.08 | 13.80 | 10.76 | 17.24 | 15.53 | 7.33 |
| 2 | 16.59 | 17.27 | 21.86 | 17.20 | 21.45 | 18.33 | 6.93 |
| 3 | 17.96 | 18.84 | | 17.69 | 20.76 | 18.94 | 6.18 |
| 4 | 19.93 | 19.95 | | 17.92 | | 22.29 | 6.38 |
| 5 | 17.84 | 19.55 | | 17.69 | 21.21 | 19.83 | 6.79 |
| 6 | 16.65 | | | | | | |
| 7 | 20.74 | | 17.50 | | | | |
| 8 | | | 16.02 | | | | |
| 9 | | | 17.53 | | | | |
| Average without Day 1 → | 18.42 | 18.90 | 18.23 | 17.62 | 21.10 | 19.85 | 6.57 |
| Weight loss, g/day → | 583 | 590 | 265*** | 409 | 908 | 953 | |

*Casec
**During the protein period, subject consumed 400 kcal as egg white, turkey, and/or crab; during the carbohydrate period, diet consisted of 400 kcal from dried fruits and jelly candy.
***Data for 12 days, the last 3 of which included some dietary carbohydrate. Interval data are not available.

to two factors: previous customary nitrogen intake and the presence of reserve carbohydrate in the form of tissue glycogen. Excluding the first test day, the four women excreted 17.62 to 18.90 g per day, on the average, and the man, 21.10 g. Another man, who ate egg albumen and muscle meats, also excreted more nitrogen than did the women, 19.85 g per day. Data on fecal excretion are not available but, assuming the normal value, output exceeded intake by about 6 to 7 grams per day for the women, and 8 to 10 grams for the men. This contrasts with the much lower value of 2 grams per day in the obese subjects (v.s. ~~V.S.~~). The one subject for whom comparative data are available in this study, M, excreted only 6.6 g per day when consuming daily only 400 kcal as carbohydrate, predicting net loss of nitrogen amounting to about 8 grams per day. Thus there is no evidence for a sparing effect on body nitrogen by consuming protein at this severely limited caloric intake.

Body weight loss was about the same for the women of this study as for the grossly obese subjects (Tables 9 and 29). Weight loss was greater for the male subjects.

## Blood Constituents

Values for relevant blood constituents are given in Table 30. Blood urea nitrogen rose in conjunction with consumption of the low-calorie protein diet, and was quite elevated (in relationship to the intake) in subjects C, N, and Bo. Serum uric acid was also elevated, and subject Bo experienced an acute attack of gout at the end of the study. Blood lipid levels were not consistently affected by dietary treatment.

Table 30

BLOOD CONSTITUENTS OF NORMAL SUBJECTS BEFORE AND AFTER A PERIOD OF DIETARY RESTRICTION*

| | Female Subjects | | | | Male Subjects | |
|---|---|---|---|---|---|---|
| | Ba | C | G | N | BO | M* |
| | Uric acid, mg/100 ml | | | | | |
| Initial | 3.3 | 4.9 | 4.2 | 4.6 | 6.0 | 5.9 |
| Final | 4.4 | 6.1 | 5.0 | 5.2 | 7.2 | 6.3* |
| | Blood urea nitrogen, mg/100 ml | | | | | |
| Initial | 13.5 | 15.5 | 13.0 | 16.5 | 18.0 | 14.5 |
| Final | 16.5 | 25.0 | 14.5 | 22.0 | 24.5 | 11.5* |
| | Serum total cholesterol, mg/100 ml | | | | | |
| Initial | 210 | 227 | 230 | 219 | 250 | 212 |
| Final | 176 | 196 | 218 | 230 | 216 | 200* |
| | Serum triglycerides, mg/100 ml | | | | | |
| Initial | 52 | 100 | 22 | 43 | 152 | 176 |
| Final | 62 | 86 | 50 | 58 | 98 | 72* |

*After 5 to 12 days of eating 100 g casein/day for all subjects except M, who was tested after the low-calorie carbohydrate period.

## REFERENCES


1. Owen, O. E., J. M. Sullivan and G. F. Cahill, Jr., *Clin. Res.*, 14: 351, 1966.
2. Lecocq, F. R., and J. J. McPhaul, *J. Metab., Clin. Exptl.*, 14: 186, 1965.
3. Drenick, E. J., *Arthritis and Rheum.*, 8: 988, 1965.
4. Bloom, W. L., *Arch. Int. Med.*, 109: 26, 1962.
5. Ullrich, K. J., and D. J. Marsh, *Ann. Rev. Physiol*, 25: 91, 1963.
6. Tolstoi, E., *J. Biol. Chem.*, 83: 753, 1929.
7. McClellan, W. S., and E. F. Dubois, *J. Biol. Chem.*, 87: 651, 1930.
8. Gamble, J. L., Harvey Lecture Series, 42: 247, 1946.
9. Cathcart, E. P., *J. Physiol*, 39: 311, 1909.
10. Munro, H. N., *Physiol. Rev.*, 31: 449, 1951.
11. Calloway, D. H., and H. Spector, *Am. J. Clin. Nutr.*, 2: 405, 1954.
12. Reifenstein, E. C., Jr., F. Albright and S. L. Wells, *J. Clin. Endocrinol.*, 5: 367, 1945.
13. Chinn, K. S. K., *J. Nutrition*, 90: 323, 1966.

## APPENDIX A

### HISTORIES OF SUBJECTS OF EXPERIMENT NO. 6

0601.

17 year old white female, single

Date of birth: March 6, 1946

Height: 181 cm (71.25 in)

Weight before study: 123.4 kg (271.6 lbs)

Subject has resided in Berkeley all her life.

Education: currently at high school

Family history: parents divorced when subject was a baby; father age 50; mother age 51, obese; no siblings

Systemic review: heart systolic murmur, area of origin not clear. Otherwise negative

Weight history: subject weighed 8 lbs at 2 weeks of age, 16 lbs at 2 months and since then has always been chubby and overweight

Dietary habits:

Mother and daughter have irregular eating habits. The mother works from 7 a.m. to 3 p.m. and dislikes cooking. The subject eats anything in the refrigerator for breakfast, provided no preparation is involved, usually juice and/or breakfast cereal. She buys her lunch at school; hamburger or sandwich, "Coca Cola," pie, ice cream, candy bars, cookies. She probably eats more between meals than at them. The time of the evening meal varies between 4:30 p.m. and 9:00 p.m. and is home-cooked 1-2 times a week; other meals are bought out, apart from occasional meals at the home of the grandparents who eat regularly at 5:30 p.m. Very little food is kept in the house. The subject likes fruit but rarely eats it. She dislikes fish and certain meats. She takes large portions at meals, always feels hungry and eats more when she is upset, and while she watches television. She is very inactive, watches a great deal of television, goes to bed after midnight. She smokes very rarely, and never consumes alcohol.

0602.

42 year old white female, widow

Date of birth: March 13, 1924

Height: 164 cm (64.56 in)

Weight before study: 116.2 kg (255.7 lbs)

Subject moved to the Bay Area from Chicago in 1960.

Education: 3 years of college

Occupation: housewife and part-time advertising copy-writer

Family history: Father age 70; mother age 67; both obese. History of obesity in females of mother's family. Father's family all short and overweight. No siblings. Married at age 27. One son age 14, not overweight. Widowed in 1956.

Weight history:

Since the age of 18 the subject has nearly always been at least 10 lbs overweight. Between the ages of 18 and 27, when the subject married (1951), her weight fluctuated between 132 lbs and 160 lbs. In 1952 she gained 25 lbs in the first 7 months of pregnancy. At delivery she weighed 145 lbs, which increased to 160 lbs after she ceased to nurse the baby. In January, 1959, the subject weighed 211 lbs, which she had reduced, by August 1960, to 165 lbs. Since 1961 her weight has gradually increased, and the rate of increase has been very rapid in the past 6 months.

Dietary habits:

The subject enjoys cooking for herself and her son but dislikes cleaning up after a meal, consequently they eat out 3 or 4 evenings a week. The eating pattern is erratic. She rises at 7 a.m., has fruit juice or no breakfast, may eat a sandwich at noon but often eats nothing until 3-4 p.m. The evening meal is usually taken between 5 p.m. and 7 p.m. but can be as late as 10 p.m. The subject prepares potatoes, rice and fried foods for her son and eats them herself in spite of continual resolutions not to do so. She rarely eats salads, and is fond of cookies, cakes and candies, and places great emphasis on the special heavy and sweet breads she purchases.

The subject's main problem is compulsive evening eating, which she believes is a result of emotional stress and is a sex-substitute.

She suffers from some insomnia, and regularly eats sandwiches during the night.

She smokes approximately 20 cigarettes a day and occasionally consumes alcohol.

When younger she walked, swam and played tennis regularly but her activities have declined in recent years during which she has had a car.

0603.

46 year old white female, married

Date of birth: February 5, 1920

Height: 162.5 cm (63.97 in)

Weight before study: 140.8 kg (309.7 lbs)

Subject moved to the Bay Area from Salt Lake City in 1946.

Education: 2-1/2 years of college

Occupation: housewife

Family history: Father age 77; mother age 66 recently found to be diabetic; 1 brother not living; 1 sister age 39. No obesity. Husband age 56, 3 children, none obese.

Weight history:

As a child the subject was always of average weight and weighed 120 lbs at age 20 when she married. During her first pregnancy at age 22 she gained 40 lbs, 20 lbs of which she retained after the birth of the baby. During her second pregnancy she similarly gained another 20 lbs. Since her third pregnancy 10 years ago her weight has constantly increased. She has tried to lose weight many times during the past 10 years but has always regained more than she lost. Her movements have slowed markedly in the past year.

Dietary habits:

The subject greatly enjoys cooking for her family, using many Greek dishes which incorporate oil. She cooks breakfast for her husband and son, usually takes only fruit juice herself. She is very conscious of her food intake and finds that once she starts to eat during the day she cannot make herself stop; she either eats nothing or too much. She often has no lunch, but may eat several sandwiches around 4 p.m. when she prepares snacks for the children, before she begins to prepare the evening meal. She enjoys meat and vegetables and eats large portions. She prefers a substantial sandwich to a sweet dessert. She neither smokes nor consumes alcohol.

The subject's husband has done all the family food shopping for many years.

0604.

55 year old white female, married

Date of birth: August 9, 1910

Height: 167.5 cm (65.9 in)

Weight before study: 136.7 kg (300.6 lbs)

The subject moved to the Bay Area from Chicago in 1941.

Education: high school

Occupation: housewife. The subject has worked intermittently for years as a nurse, mainly of mental cases, in private homes.

Family history: Father died at age 82; mother dead; 1 brother age 53; 2 sisters ages 61 and 50 all living and well. All became overweight during their forties. Married at age 28. Husband age 60, not obese.

Systemic review: The subject is mildly diabetic and is on "Dymelor" 3/day. She had not had a medical check-up for about a year, but was previously told that weight loss would improve her condition.

Weight history:

The subject remembers always being well-built. At age 18 she weighed 127 lbs, at age 25, 165 lbs. Since then she has gradually gained weight, although she has made many attempts to reduce her weight. Three years ago her husband bought a car and the subject gained 75 lbs in the following 18 months. Since that time she has made no attempt to lose weight.

Dietary habits:

The subject likes to sleep until 11 a.m. and eats a breakfast of 2 eggs, bacon, and at least 2 slices of toast. She usually has a large meal with soup, salad, meat, spaghetti or potatoes, and fruit at about 4 p.m. In the evening she is continually eating; crackers, fruit, milk, cheese, sandwiches, but she very rarely eats cookies or candy. She may eat a potful of cereal or rice pudding, or a loaf of bread, particularly when told by someone else to eat less. She likes to cook for her husband, who is thin, and enjoys shopping for food, which she considers to be a hobby.

Until the last three years the subject was always active and did a great deal of swimming and walking.

She smokes up to 4 cigarettes a day and never consumes alcohol.

0605.

37 year old white female, married

Date of birth: October 2, 1928

Height: 164 cm (64.56 in)

Weight before study: 133.8 kg (294.4 lbs)

The subject has lived in the Bar Area all her life.

Education: high school

Occupation: housewife. The subject has also been working virtually full-time in a family-owned beauty shop.

Family history: Father died at age 79 of kidney disease; mother age 66; neither obese; 2 brothers ages 40, 41, one has a tendency to be overweight. Husband age 38, average weight; 2 daughters ages 7 and 5, the elder is heavy for her age.

Weight history:

At age 18 the subject weighed 145 lbs, at age 25 when she married she weighed 170 lbs. She has had 3 pregnancies, one of which terminated in a miscarriage after 3-1/2 months. The last baby weighed 12 lbs at birth. Over the last 14 years the subject's weight has continually increased. She has started many reducing diets, but has regained more than she lost after every attempt. Her present weight is the maximum she has ever weighed.

Dietary habits:

The subject eats a breakfast of 3 slices of toast with honey and butter, tea or chocolate at 7:30 a.m. When she is at home she eats lunch but on most days when she is at work, which includes Saturdays, she eats nothing all day. This has been a habit for years. She is on her feet all day and eats nothing for 8-12 hours. In recent months the subject's mother-in-law has done the family cooking, although the subject makes all the food purchases. She and her husband eat a meal of meat, salad and dessert between 10 p.m. and midnight and watch TV for an hour before bed. The subject likes to cook. She likes cookies and candy but rarely eats them, unless she is waiting for her evening meal. She feels that she is efficient in all her movements.

## APPENDIX B

### Analytical and Clinical Methods

A. Laboratory Methods Used in the Human Nutrition Research Unit

Calcium:

Analytical Methods for Atomic Absorption Spectrometry (Solid Materials), pg. Ca 2, The Perkin-Elmer Corporation, Norwalk, Conn. (1964).

Catecholamines:

Manual of Fluorometric Clinical Procedures, pg. 13, Turner Instrument Co., Palo Alto, Calif. (1962).

Chlorides:

"Automatic Titrations with Aminico-Cotlove Automatic Chloride Titrator," Cat. No. 4-4420B Instruction No. 751-C, American Instrument Co. (1964).

Cotlove, E., and H. H. Nishi, *Clin. Chem.*, 7: 285 (1961).

Citric Acid:

Methods in Enzymology, Vol. III, Academic Press (1957), pg. 426.

Creatinine and Creatine:

Henry, Richard J., Clinical Chemistry, Harper and Row (1964), pg. 292.

Hydroxyproline:

Prochop, Darwin J., and Sidney Udenfriend, *Anal. Biochem.*, 1: 228 (1960).

Magnesium:

Analytical Methods for Atomic Absorption Spectrometry (Liquid Materials), pg. Ca 6, The Perkin-Elmer Corporation, Norwalk, Conn. (1964).

Nitrogen:

Micro-Kjeldahl modification of Block, Richard J., and Kathryn W. Weiss, Amino Acid Handbook, Charles C. Thomas, Springfield, Ill., pg. 11 (1956), using $H_2SO_4$ as digestion mixture, selenized Hengar granules as catalyst, and 4 percent $H_3BO_3$.

Phosphorus:

Colowick, Sidney P., and Nathan O. Kaplan, Methods in Enzymology, Vol. III, Academic Press, Inc., N.Y., pg. 843 (1957).

Potassium:

Analytical Methods for Atomic Absorption Spectrometry (Liquid Materials), pg. Ca 6, The Perkin-Elmer Corporation, Norwalk, Conn. (1964).

Sodium:

Analytical Methods for Atomic Absorption Spectrometry (Liquid Materials), pg. Ca 6, The Perkin-Elmer Corporation, Norwalk, Conn. (1964).

Thiamine:

Consolozio, C. Frank, and Robert E. Johnson, "Biochemical and Dietary Procedures, U.S. Army Medical Research and Nutrition Laboratory Report #242 (1960).

Urea:

Coulomke, J. J., and L. Faureau, *Clin. Chem.*, 9: 102 (1963).

Uric Acid:

Dermatube-U Kit (Enzymatic, Uricase), Worthington Biochem. Corp. (1965).

Xanthurenic Acid:

Modification of Satoh, Kiyoo, and J. M. Price, *J. Biol. Chem.*, 230: 781 (1958), using Turner fluorometer with primary filter #110-818 (7-60) and secondary filter #110-818 (24-12).

Total Ketone Bodies:

Michaels, G. D., S. Margen, G. Liebert, and L. W. Kinsell, Studies in fat metabolism. I. The colorimetric determination of ketone bodies in biological fluids. *J. Clin. Invest.*, 30: 483 (1951), as modified by: Britton, H. G., The chemical estimation of ketone bodies, *Analyt. Biochem.*, 15: 261 (1966).

B. <u>Methods Unique to Blood Analyses</u>

Ammonia:

W. Muller-Beissenherz und H. Keller, *Klin. Wochenschs*, <u>43</u>: 43 (1965).

Bilirubin:

Molloy, H. T., and K. A. Evelyn, *J. Biol. Chem.*, <u>119</u>: 481 (1937).

Cholesterol:

Technicon Auto-Analyzer, Technion Co., Chauncey, N.Y., 1963.

Zlatkis-Zak reaction on isopropanol extract of serum.

Zlatkis, A., B. Zak, and A. J. Boyle, *J. Lab. and Clin. Med.*, <u>41</u>: 486 (1953).

Electrophoresis, Protein:

Cellulose actetate, Ponceau R. stain. Resolved fractions eluted and color determined at 520 mμ in Beckman D. U. Spectrophotometer.

Glucose:

Technicon Auto-Analyzer, Technion Co., Chauncey, N.Y., 1963.

Modification of Hoffman, W. S., *J. Biol. Chem.*, <u>120</u>: 51 (1937).

Glutamic Pyruvic Transaminase, Serum:

Technicon Auto-Analyzer, Technicon Co., Chauncey, N.Y., 1963.

Unpublished method determination of pyruvate formed from d,l-alanine by use of salicylaldehyde.

Protein, Total Serum:

Weichselbaum, T. E., *Amer. J. Clin. Path.*, <u>7</u>: 40 (1946).

Protein-Bound Iodine:

Dry Ash Method - Barker, S. B., <u>Standard Methods of Clinical Chemistry</u>, Vol. 3, Academic Press (1961), pg. 167.

Totally automated method - Technicon Auto-Analyzer, Technicon Co., Chauncey, N.Y., 1963.

Uric Acid:

Reduction of phospho-tungstic acid, Technicon method N-13a, Technicon Co., Chauncey, N.Y., 1963.

Urea Nitrogen:

Technicon Auto-Analyzer, Technicon Co., Chauncey, N.Y., 1963.

Modification of Skeggs, L. T., *Amer. J. Clin. Path.*, 28: 311 (1957), using carbamido-diacetyl reaction applied to urea.

C. Gas Measurements

Flatus:

Fisher gas partitioner - dual column gas chromatograph.

Respiratory:

Hydrogen - Wilkens aerograph detector Model 600-C.

Methane - Carad flame ionization unit.

Oxygen - Beckman paramagnetic oxygen analyzer.

Carbon Dioxide - Pulmo analyzer - Thermal analyzer (Godart, Holland).

D. Miscellaneous

Circulating Blood Volume:

Method for Evans Blue - Warner-Chilcott Division, Morris Plaines, N.J.

- Henry, R. J., Clinical Chemistry Principles and Techniques, Harper and Row (1964), pp. 899-902.

Total Body Water:

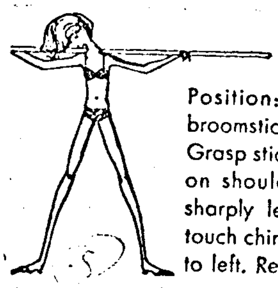
Boling, E. H., *Annals N.Y. Acad. Sci.*, 110: 246 (1963).

Body Composition:

Keys, Ancel, and J. Brozek, "Body Fat in Adult Man," *Physiol. Rev.*, 33: 245 (1953).

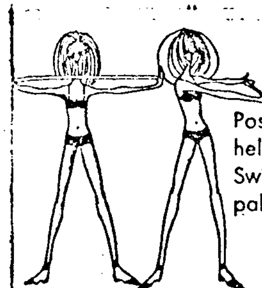
Behnke, R. N., "Anthropometric Evaluation of Body Composition Throughout Life," *Annals N.Y. Acad. Sci.*, Part 2: 450 (1963).

# Daily Exercises



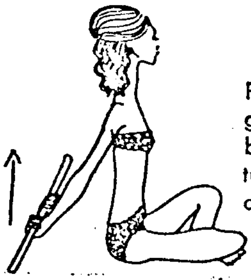
Position: Hold smooth pole, broomstick length, on shoulders. Grasp stick near ends. Rest stick on shoulders, then turn head sharply left to right, trying to touch chin to stick. Reverse right to left. Repeat swings 15 times.

1.



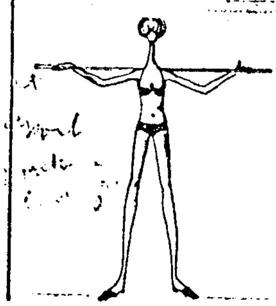
Position: Stand with stick ends held between palms of hands. Swing arms from side to side, palming stick firmly. 20 times.

2.



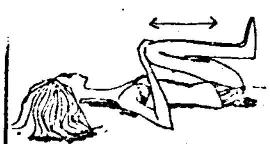
Position: In seated position, grasp stick with palms up behind back, hands close together on stick. Push stick directly up and down 30 times.

3



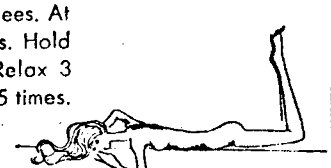
Position: Stand with stick on shoulders behind head, arms curved behind stick, hands dropped forward. Bend from side to side, up and down, then twist from side to side. 30 times.

4



Position: Lie flat on bed. Draw both knees up. Place hands on knees, palms down. Press down on knees, but force knees to resist. Let arms win out, finally, until legs are pushed out straight. 10 times. Breathe between each resistance contest.

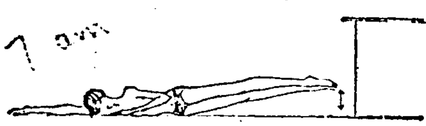
5



Position: Lie on stomach, on floor or bed. Place a book between lower thighs. Squeeze book with thighs. Flex knees. At the same time, lift knees. Hold elevation 6 seconds. Relax 3 seconds. Repeat about 5 times.

6

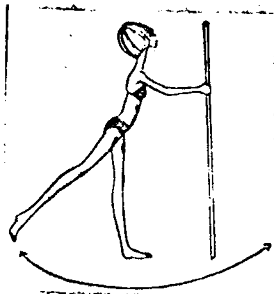
Position: Lie on side on floor near table. Keep back straight. Lift both legs toward table top. Hold 6 seconds. Relax 3 seconds. Reverse sides. Repeat 5 times each side. Table's purpose: to provide a target.



7

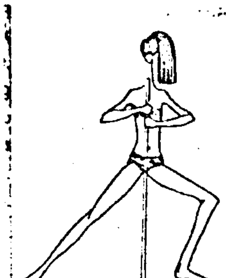
Position: Lie on bed, body-weight resting on propped elbows. Flex knees. Swing feet in wide circles, both feet circling outwards, in opposite directions from each other. Then reverse, swinging both feet in circles towards each other. 25 times for each set of foot circlings.

8



Position: Stand upright, with stick upright from floor. Hold stick with both hands and swing either leg forward and back as far as possible. Alternate left to right. Reverse. 30 times each.

9



Position: Stand with legs wide apart facing upright stick. Hold stick with both hands. Keeping back straight, knee-dip deeply to left, then deeply to right. Keep buttocks tucked in. 12 times.

10



VOGUE, April 15, 1966, p 116.